Published in final edited form as:
*J Hazard Mater*. 2023 September 05; 457: 131738. doi:10.1016/j.jhazmat.2023.131738.

# Microbial Functionalities and Immobilization of Environmental Lead: Biogeochemical and Molecular Mechanisms and Implications for Bioremediation

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## Abstract

The increasing environmental and human health concerns about lead in the environment have stimulated scientists to search for microbial processes as innovative bioremediation strategies for a suite of different contaminated media. In this paper, we provide a compressive synthesis of existing research on microbial mediated biogeochemical processes that transform lead into recalcitrant precipitates of phosphate, sulfide, and carbonate, in a genetic, metabolic, and systematics context as they relate to application in both laboratory and field immobilization of environmental lead. Specifically, we focus on microbial functionalities of phosphate solubilization, sulfate reduction, and carbonate synthesis related to their respective mechanisms that immobilize lead through biomineralization and biosorption. The contributions of specific microbes, both single isolates or consortia, to actual or potential applications in environmental remediation are discussed. While many of the approaches are successful under carefully controlled laboratory conditions, field application requires optimization for a host of variables, including microbial competitiveness, soil physical and chemical parameters, metal concentrations, and co-contaminants. This review challenges the reader to consider bioremediation approaches that maximize microbial competitiveness, metabolism, and the associated molecular mechanisms for future engineering applications. Ultimately, we outline important research directions to bridge future scientific research activities with practical applications for bioremediation of lead and other toxic metals in environmental systems.

### Keywords

Phosphate solubilizing bacteria; sulfate reducing microorganism; microbial induced carbonate precipitation; microbial remediation; lead; heavy metals

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## 1. Introduction

Environmental lead (Pb) contamination is ranked at the top of the World's Worst Pollution Problems based on a global assessment of human health risks at hazardous waste sites (Cross, 2012). Lead is highly poisonous, causing severe damage to the brain and kidneys and, ultimately, death. It has been long recognized that exposure to Pb may result in significant health impacts. About 1% of the total global burden of disease is attributed to mild intellectual disability and cardiovascular problems caused by Pb exposure (Fewtrell et al., 2004). Children are exceptionally vulnerable because their bodies absorb four to five times as much Pb as adults. Major anthropogenic activities causing Pb pollution includes Pb smelting, mining and ore processing, Pb-acid battery recycling and manufacturing. In the United States, reducing human exposure to Pb is a priority for the U.S. Environmental Protection Agency (EPA) as stipulated in the December 2018 Federal Action Plan to Reduce Childhood Lead Exposures and Associated Health Impacts (U.S. EPA, 2019a). Most recently, the emphasis is placed on reducing Pb exposures and disparities in U.S. communities (U.S. EPA, 2022).

The increasing environmental and human health concerns over Pb in the environment have stimulated scientists to search for innovative, eco-friendly, and cost-effective technologies for remediation of Pb contaminated sites. Conventional remediation methods for Pb contaminated sites rely on physical removal of material and engineering control (U.S. EPA, 2019b), yet these traditional techniques often require soil excavation and transportation, which in turn, generates high cost and energy input along with negative impacts on soil structure and existing ecosystems. In-situ site stabilization methods involve chemical addition to reduce Pb acute toxicity and bioavailability, thereby controlling the risk of Pb exposure (U.S. EPA, 2019b). Phosphate containing compounds such as rock phosphate and triple superphosphate are especially effective to immobilize Pb in contaminated sites due to the formation of highly recalcitrant Pb phosphate precipitates such as pyromorphite (Bradham et al., 2018; Scheckel et al., 2013). Carbon-based materials, including pristine or modified biochars, have been shown to be effective sorbents of Pb in both aqueous substrate and contaminated soils through a number of adsorption mechanisms (Cao et al., 2011; Cao et al., 2009; Ding et al., 2016; Uchimiya et al., 2012; Wang et al., 2019a). Additionally, bioremediation has received considerable attention as an alternative technology for Pb-contaminated sites (Muthusaravanan et al., 2018), such as sustainable biomineralization and biocement technologies (Sharma et al., 2022; Yu and Zhang, 2023) or the use of a consortia of cyanobacteria, algae or diatoms for metal recovery and restoration by leveraging their biosorption properties in industrial applications (Adey et al., 1996; Ajayan et al., 2018; Safonova et al., 2004).

The capability of microorganisms to mobilize or immobilize heavy metals is fundamental to the biogeochemical cycles of metals, providing the basis for the development of effective environmental bioremediation technologies to aid in heavy metal sequestration or removal from the environment. The functionality of microorganisms to influence and/or mediate metal mobilization or immobilization processes stems from their ability to modulate the balance of metal species between soluble and insoluble phases. Microorganisms attain an ecological advantage in Pb contaminated environments, primarily due to extracellular and intracellular strategies that either exclude or sequester Pb in nontoxic forms (Jaroslawiecka and Piotrowska-Seget, 2014; Naik and Dubey, 2013; Pan et al., 2017). Mobilization of metals can be achieved by protonation, chelation, and chemical transformation; however, immobilization occurs by precipitation or crystallization of insoluble organic or inorganic compounds or by sorption, uptake, and intracellular sequestration. In such a context, solubilization processes may enable removal from solid matrices, such as soils, sediments, dumps, and industrial wastes. Alternatively, immobilization processes may enable metals to be transformed in situ into insoluble and chemically inert forms and are also applicable to removing metals from aqueous solution.

Immobilization of toxic Pb into insoluble precipitates is an important strategy to reduce Pb bioavailability and toxicity to acceptable levels. Microbial mediated precipitates such as Pb phosphate, sulfide, and carbonate derivatives, can sequester bioavailable Pb in water, soil, and sediment. Once formed, these recalcitrant compounds render less Pb bioavailability or even reduce or eliminate its toxicity, thereby remediating the affected media. Furthermore, because precipitates can be more easily recovered than unbound Pb, these same mechanisms can be engineered for industrial bioremediation purposes. Phosphate solubilizing bacteria (PSB), sulfate reducing microorganisms (SRM), and carbonate synthesizers for microbial induced carbonate precipitation (MICP) are the primary microbial groups that can immobilize Pb in the presence of other heavy metals in a variety of environmental media (Fig. 1). In recent years, much research has been done to characterize these microorganisms' isolation and selection, taxonomy, functionalities, and mechanisms for immobilization of Pb in the environment (Fuchida et al., 2020; He et al., 2019; Neculita et al., 2008; Niu et al., 2018; Park et al., 2011a; Park et al., 2011b; Teng et al., 2019; Yin et al., 2020b; Zheng et al., 2019). It is well documented that the efficiency of microbial-mediated immobilization of environmental Pb depends on not only the particular taxa but also the ambient environment of contaminated sites (Sevak et al., 2021; Shan et al., 2021). In addition, state-of-the-art research on these microbial mediated processes emphasizes practical applications of bioremediation. For example, biogenic compounds, such as schwertmannite (iron-oxyhydroxysulphate) precipitated by *Acidithiobacillus ferrooxidans* in high sulfate/Fe acidic environments, immobilized the heavy metals Pb, As, and Cd (Chai et al., 2016; Liao et al., 2009; Min et al., 2017). Biogenic iron (oxyhydr)oxide-nano-silica composites removed aqueous Sb ($Sb^{+3}$ and $Sb^{+5}$) and Cr (Seo and Roh, 2015; Xu et al., 2022a). The sulfate reducing bacterium, *Desulfovibrio desulfuricans* subsp. *desulfuricans*, mediated FeS-kaolin formation which effectively immobilized multiple heavy metals including Cd, Pb, Cu, Zn, As, and Sb (Xu et al., 2022b). *Clostridium* sp. produced bio-magnetite and bio-Fe-S/siderite, both of which immobilized Cr and have the potential to bind Pb (Seo et al., 2013). Biocement technologies also are under investigation to incorporate various waste materials such as sandstone waste (Sharma et al., 2022), carbide sludge (Yu and Zhang, 2023), or sulfate reducing bacteria containing granules (Chetty et al., 2022). Practical applications of microorganism-based environmental remediation may benefit from a systematic synthesis of the existing literature on this subject.

The objective of this work is, through a thorough review of the existing literature, to understand the important microbiological processes of environmental Pb immobilization which are of significance in actual or potential applications in environmental remediation. Specifically, we focus on microbial functionalities of phosphate solubilization, sulfate reduction, and carbonate synthesis in mediating the immobilization process of environmental Pb. Ultimately, we outline some important future directions to bridge the scientific research with practical applications for bioremediation.

## 2. Immobilization of Lead by Phosphate Solubilizing Bacteria

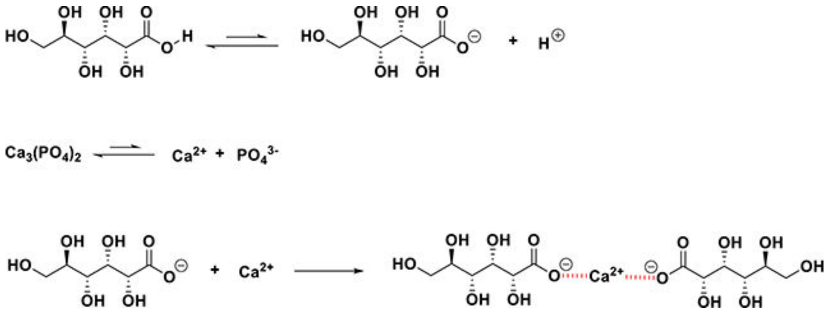
The term phosphate solubilizing bacteria (PSB) refers to diverse beneficial bacteria capable of mobilizing P from insoluble compounds, resulting in elevated levels of available phosphate in the environment. Since the pioneering work of Pikovskaya (1948) that linked mobilization of P in soils with vital activities of some microbial species, PSB and their roles in P cycling have been a focal research area with a primary emphasis on identifying strains for agronomic applications as inoculants to increase soil available P content by releasing P bound by Al, Fe, and Ca (Rodríguez and Fraga, 1999). Therefore, PSB traditionally refer to bacteria that solubilize inorganic P compounds. Phosphate solubilizing bacteria are widely distributed in soils, water, and sediments (Liu et al., 2014; Zhang et al., 2015). While microbial communities vary significantly with the ambient environment, up to 50% of the total number of bacteria in soil are PSB, concentrated in the rhizosphere where they are metabolically active. PSB are especially active under abiotic stress such as drought, low or high pH, salinity, and temperature (Sukweenadhi et al., 2015). In the context of phosphate-based remediation of Pb-contaminated soils and wastes (Scheckel et al., 2013), scientists have paid increasing attention to the value of PSB in immobilizing Pb in soils and water (Pagnout et al., 2018; Park et al., 2011a; Park et al., 2011b; Teng et al., 2019; Yuan et al., 2017; Zhang et al., 2019a). A large number of studies isolated PSB strains that convert either inorganic or organic P into reactive phosphate that binds Pb (Table 1). Some examples of such PSB include *Bacillus subtilis* (Bai et al., 2014), *Pantoea* sp. (Chen and Liu, 2019; Park et al., 2011b), *Enterobacter* sp. (Chen et al., 2019; Li et al., 2018; Park et al., 2011a), *Acinetobacter pittii* (Wan et al., 2020), *Serratia marcescens* (Zhu et al., 2019a), *Leclercia adecarboxylata* and *Pseudomonas putida* (Teng et al., 2019). Fungal strains, such as *Penicillium chrysogenum*, also have been found to be capable to solubilizing phosphate to form Pb-phosphate minerals (Povedano-Priego et al., 2017). In this section, we summarize the mechanisms of phosphate solubilization used by PSB in the context of immobilization of Pb and the processes and practical considerations of using PSB for remediation.

### 2.1 Mechanisms of phosphate solubilization

Total phosphorus (P) content in soils is generally less than 0.1% and only 0.1% of total P is reactive or biologically available. Most P in soils is insoluble, bound by Fe and Al in acidic soils and Ca in alkaline soils. Phosphate solubilizing bacteria play a key role in biogeochemical transformations of P in soils. Insoluble P exists in soils in two forms: (i) inorganic P ($P_i$) as Al-, Fe-, or Ca-phosphates or sorbed by Al, Fe, Ca oxides, and (ii) organically bound P ($P_o$) as soil organic matter and plant/animal residues (Fig. 2). PSB employ different strategies to convert insoluble forms of P into reactive forms (primarily three species of orthophosphate). The literature generally supports two primary mechanisms of microbial mediated P solubilization: (1) $P_i$ -solubilization by secreted organic acids or hydrogen ions that dissolve $P_i$ compounds; and (2) $P_o$-mineralization by enzymatic action such as extracellular phosphatase to mineralize $P_o$ compounds (Fig. 2). Most PSB are specific to solubilization of either $P_i$ or $P_o$; yet a PSB isolate from alfalfa rhizosphere soil, *Pantoea sp.* S32, possessed high P dissolving capacity for both $P_i$ and $P_o$ (Chen and Liu, 2019). An *Enterobacter cloacae* strain also used both mechanisms (organic acid production and phosphatase action) to mobilize P and precipitate Pb to form pyromorphite (Park et al., 2011b).

### 2.1.1 Production of organic acids—

The formation of low molecular weight organic acids during microbial metabolism of organic carbon is a key process involved in solubilization of inorganic phosphate. Organic acids released by PSB act as good chelators of divalent cations of $Ca^{2+}$ coupled with the release of phosphate from insoluble complexes (Sashidhar and Podile, 2010). Organic acids also may form soluble complexes with other metal ions that are co-complexed with insoluble P, thereby releasing the P moiety. In addition, inorganic soil phosphates of Ca, Fe, and Al can be solubilized via acidification by organic acids. The foremost mechanism for mineral phosphate solubilization by Gram-negative PSB is through the production of organic acids related to the dissolution of mineral glucose to gluconic acid. These reactions can be represented as follows using gluconic acid as an example:



The other commonly produced organic acids by PSB include acetic, pyruvic, fumaric, succinic and citric acids (Bolan et al., 1994). For example, Schneider et al. (2010) suggested that citric acid and oxalic acid produced by *Aspergillus niger* mobilized P from phosphate rock. Xu et al. (2019) found positive correlations between concentrations of soluble phosphate and citric (r = 0.923), gluconic (r = 0.926), and pyruvic acids secreted by *Pantoea ananatis* HCR2 and *Bacillus thuringiensis* GL-1. Pande et al. (2017) noted production of glucuronate, formate, and citrate by *Alcaligenes aquatilis* and *Burkholderia cepacia*. Chen et al. (2006) demonstrated the secretion of citric, gluconic, lactic, succinic and propionic acids by several PSB strains (*Arthrobacter, Bacillus, Serratia, Chryseobacterium, Pseudomonas, and Delftia*), which exhibited the ability to solubilize considerable amounts of tricalcium phosphate.

The low molecular weight organic acids produced by PSB contain functional groups such as hydroxyl and carboxyl that chelate cations bound to phosphate (Fe and Al in acid soils, Ca in alkaline soils) and release soluble phosphate (Sharma et al., 2013). Type and position of the ligand in addition to acid strength determine effectiveness in the solubilization process (Kpomblekou-a and Tabatabai, 1994). Carboxylic anions produced by PSB, have especially high affinity to Ca and solubilize more P than acidification alone (Staunton and Leprince, 1996). Chelation of metal cations is greatly influenced by the molecular structure of organic acids, particularly by the number of carboxyl and hydroxyl groups. Complexation of cations becomes an important mechanism in P solubilization if the organic acid structure favors complexation (Fox et al., 1990). Exopolysaccharides and siderophores secreted by PSB may function similarly to release phosphate. Phosphorus desorption potential decreases with increasing stability of Fe - or Al - organic acid complexes in the order: citrate > oxalate > malonate / malate > tartrate > lactate > gluconate > acetate > formate (Ryan et al., 2001).

### 2.1.2 Reduction of pH

**2.1.2 Reduction of pH—**Many PSB cause reduction in the pH of their surrounding environment either by $H^+$ extrusion or by secretion of organic acids. Thus, phosphate solubilization by lowered pH can be the result of the combined effect of $H^+$ extrusion and organic acids production. However, excretion of $H^+$ may occur in the absence of organic acid production such as in N assimilation and respiratory $H_2CO_3$ production (Arvieu et al., 2003). The decrease in pH is an important process regulating the solubilization of $Ca_3(PO_4)_2$ as well as the enzyme activity involved in biosynthesis and growth. Protonating phosphate is the major pathway enhancing the dissolution of $Ca_3(PO_4)_2$:

$$Ca_3(PO_4)_2 \xrightarrow{dissolution} Ca^{2+} + PO_4^{3-}$$

$$H^+ + PO_4^{3-} \rightarrow HPO_4^{2-}$$

$$H^+ + HPO_4^{2-} \rightarrow H_2PO_4^-$$

Negative correlations between pH and P solubilized have been reported by several researchers (Park et al., 2011b; Teng et al., 2019). Park et al. (2011b) indicated that 88.4% of variability in P solubilization can be explained by pH and organic acid concentration. Kumar et al. (2008) isolated a metal tolerant plant growth promoting bacterium (*Enterobacter* sp.) which decreased the pH of the growth medium from 7 to 2, thereby achieving the maximum P solubilization of 229 mg/L. Similarly, the effectiveness of phosphate rock as a P source depends mainly on pH in acid soils ($pH < 6.5$) and as a metal immobilizing agent in acid mine wastes (Harris and Lottermoser, 2006). Thus, effectiveness of rock phosphate amendment for Pb immobilization in soils can be enhanced by adding inorganic or organic acids (Cao et al., 2009; Park et al., 2011b).

It should be noted that pH reduction induced by growth of PSB may inhibit Pb-phosphate formation though the immobilized Pb in solution can increase with reduction in solution pH due to solubilization of phosphate. A near neutral or alkaline pH is most conducive for Pb-phosphate precipitation, which is not favored with decreasing or increasing solution pH (Kopittke et al., 2008). Therefore, it is important to maintain favorable solution pH for both solubilization of P and immobilization of Pb with P compounds. Most soils have a buffering capacity that resists pH change. Thus, the effect of PSB in lowering pH as measured in growth media can be diminished when applying in soils.

#### 2.1.3 Enzymatic actions through phosphatases—

PSB can produce or release either acidic or alkaline phosphatases to convert organic P $(P_o)$ into the soluble inorganic form $(P_i)$ to immobilize Pb and/or augment plant growth (Tarafdar and Claassen, 1988; recently reviewed by Antoun, 2012; Barea and Richardson, 2015; Bi et al., 2018):

$$Organic\ \ phosphate \xrightarrow{phosphatase} PO_4^{3-}$$

These phosphatase enzymes hydrolyze ester phosphate bonds to release phosphate while high-molecular-weight organic compounds are dissociated into low-molecular-weight compounds (Pereira and Castro, 2014). Phytase enzymes, commonly detected and characterized in fungal species, hydrolyze phospho-monoester bonds in phytic acids or phytates (salt form), which are the major pools of phosphorus in plant tissues. Phosphonatase and C-P lyase hydrolyze phosphonates that contain a characteristic carbon–phosphorus (C–P) bond into hydrocarbons and phosphate ions.

Phosphatase enzymes are active over a range of environmental pH. Alkaline materials such as $NH_3$ can be produced when PSB break down proteins and amino acids, a biochemical process called ammonification (Chen et al., 2019). In this case, releases of phosphate from organic material are accompanied by a pH increase. For example, a PSB stain, *Serratia marcescens* OPDB3–6-1, can produce soluble phosphate and alkaline matter during the degradation of organic phosphorus compounds (Zhu et al., 2019a). Acid phosphatase activity also was found to be positively correlated to solubilized P, serving as an indicator for organic P mineralization (Behera et al., 2014). However, acid phosphatase would not act directly on inorganic P solubilization. Instead, it may participate in lowering the pH by the dephosphorylating action and the production of organic acids (Achal et al., 2007).

#### 2.1.4 PSB induced P cycling gene expression—

Phosphate solubilization has been linked with changes in P-cycling gene abundance in soils. Phosphorus mobilizing genes, such as *phoX, phoA, and phoD* encoding alkaline phosphatase, *ptp* encoding protein tyrosine phosphatase, *hap* encoding histidine acid phosphatase, *bpp* encoding b-propeller phytase, *cphy* encoding cysteine phytase, *gcd* encoding glucose dehydrogenase, and *pqq* encoding pyrroloquinoline-quinone synthase, are involved in varying pathways of P-cycling and production of Pi (Bi et al., 2018; Huang et al., 2009; Neal et al., 2017). Wan et al. (2020) were among the first to relate $P_i$ solubilization and Pb immobilization with changes in P cycling genes. In their experiment, PSB *Acinetobacter pittii* gp-1 transformed insoluble Ca-bound phosphate into soluble P, and this was correlated to expression of *pqq* and *gcd* genes and Pb immobilization. They further noted that Pb immobilization efficiency was positively correlated with *gcd*- and *bpp*-harboring bacterial abundance, suggesting that these bacteria might be responsible for Pb immobilization. A slight increase in the abundances of organic P-cycling-related *bpp*- and *phoD*-harboring bacterial communities occurred as the indigenous bacterial community changed. Through metagenomic analysis, Liang et al. (2020) also identified a novel PSB *gcd* gene which had an important role in driving the enhancement of soil P cycling following the restoration of a degraded heavily mined site. The abundance of *pqq* and *gcd* genes in highly contaminated environments suggests that bacteria harboring these genes also can develop heavy metal resistance strategies.

### 2.2 Processes of Pb immobilization mediated by PSB

Immobilization of Pb mediated by PSB is a complex process and can involve both extracellular and intracellular mechanisms. Biomineralization and biosorption are the predominant processes involved in Pb immobilization after soluble P levels are elevated by PSB. Biosorption describes the adherence of Pb to microbial cells and Pb biomineralization is the formation Pb-containing minerals. It should be noted that these two processes often occur concurrently. In biomineralization, bacterial cells may serve as nucleation sites to support the formation of Pb-P crystals, which resembles a self-assembly process involving first dissolution of small particles and then growth of the mineral crystals. From a remediation standpoint, interactions of Pb, P on the microbial cell surface improve the removal rate and makes the mineralized products more stable and compact.

**2.2.1 Pb biomineralization—**Various Pb-P minerals have been identified as PSB-related through studies with modern analytical techniques such as X-ray diffraction analysis (XRD), scanning electron microscope (SEM), and x-ray absorption near-edge structure (XANES) or X-ray absorption fine structure (XAFS) spectroscopy using synchrotron radiation. Pyromorphite-type Pb-P minerals are perhaps the most commonly observed biomineral precipitate. The mineral phase of pyromorphite [$Pb_5(PO_4)_3X$, where X = F, Cl, Br, OH] is known as the most stable Pb mineral occurring in the terrestrial environment. Chloropyromorphite has a widely cited solubility product of $K_{sp} = 10^{-84.4}$ based on the reaction:

$$Pb_5(PO_4)_3Cl_{(s)} = 5Pb^{2+} + 3PO_43^- + Cl^-$$

Formation of a specific mineral depends on the soil environment or the experimental conditions, especially ionic composition and soil pH. For example, PSB *Pantoea ananatis* and *Bacillus thuringiensis* were assayed in broth medium containing Pb and rock phosphate and produced Pb-P compounds identified as pyromorphite [$Pb_5(PO_4)_3Cl$] and hindsdalite [$PbAl_3PO_4SO_4(OH)_6$] (Xu et al., 2019). Co-formation of hydrocerussite [$Pb_3(CO_3)_2(OH)_2$] suggested the influence of hydroxides and carbon dioxides. Acetate and malonate, synthesized by *Leclercia adecarboxylata* and *Pseudomonas putida*, were associated with Pb precipitation as hydroxylpyromorphite [$Pb_5(PO_4)_3OH$] and chloropyromorphite (Teng et al., 2019). *Bacillus megaterium* isolated from soil decomposed phosphate-containing organic compounds in culture medium containing $Pb^{2+}$ at 100–500 mg/L and formed $Pb_3(PO_4)_2$ stable minerals such as hydroxylpyromorphite [$Pb_5(PO_4)_3OH$] (Zhang et al., 2019b). During the 72-h experiment, the rate of crystallization induced by this bacterium and the shapes of the crystals formed varied, suggesting that Pb-P minerals crystalize with time.

Phosphatase activity also may serve as an indicator for Pb biomineralization. In *Citrobacter freundii* and *Staphylococcus aureus*, phosphatase activity was detected in both Pb resistant and sensitive strains at comparable levels (Levinson and Mahler, 1998). Lead nitrate [$Pb(NO_3)_2$] induced phosphatase activity to increase by 160% in *Achromobacter xylosoxidans* and resulted in the formation of extracellular pyromorphite (Sharma et al., 2018). Organic phosphate was converted to soluble phosphate by *Bacillus megaterium* phosphatase, producing a lead phosphate [$Pb_3(PO_4)_2$] precipitate through the hydroxyl intermediate, hydroxylpyromorphite [$Pb_5(PO_4)_3OH$] (Zhang et al., 2019b; Zhu et al., 2015). Microbial produced organic acids and phosphatases contribute to the soluble P pool and formation of recalcitrant Pb precipitates (Park et al., 2011a; Teng et al., 2019; Xu et al., 2019). Clearly, multiple sequestration mechanisms follow microbial phosphate generation and Pb immobilization.

**2.2.2 Cell Pb biosorption—**The Pb biosorption process results from various functional groups presented on the bacterial cell surface or cell wall that serve as sorption sites (Bai et al., 2014; Teng et al., 2019; Yuan et al., 2017). Hydroxyl, amide, carboxyl, and phosphate groups of lipids, polysaccharides, and protein amide, alkyl chain are common functional groups in the surface of cells detected with Fourier transformed infrared spectroscopy (FTIR). Comparative FTIR spectra of bacterial cells before and after Pb adsorption reveal carboxyl, hydroxyl, carbonyl, amido, and phosphate groups as the common functional groups binding $Pb^{2+}$ on the bacterial surface (Bai et al., 2014; Teng et al., 2019). For example, Yuan et al. (2017) examined a phosphate solubilizing bacterial consortium, comprised of *Enterobacter* spp. (92.65%), *Bacillus* spp. (4.90%), and *Lactococcus* spp. (1.65%), for immobilization of soil Pb and Cd with introduction of calcium phosphate [$Ca_3(PO_4)_2$] as a source of phosphate and indicated that amide I and amide II bonds and carboxyl are active with sorption of metals.

Biosorption of Pb may involve various mechanisms including physical entrapment, ionic exchange, complexation, and precipitation. The adsorption capacity of intact living cells is generally higher than that of cell debris because intact cells have complete cell surfaces, structural integrity, and functionality, while cell debris has only a simple physical adsorption function. Bai et al. (2014) developed a multi-desorption approach using $H_2O$, 1 M $NH_4NO_3$ solution, and 0.1 M EDTA-$Na_2$ solution sequentially, to partition Pb sequestration by cells into (1) physical entrapment by the cell wall mesh structure, (2) ion exchange with cell wall polysaccharides ($K^{2+,}$ $Ca^{2+}$, $Na^{2+}$, and $Mg^{2+}$), and (3) complexation or extracellular binding to cell wall functional groups such as amide, carboxyl and phosphate groups. The remaining fraction that was not desorbed by EDTA was attributed to intracellular accumulation inside the cells. These authors noted that 8.5% was physically entrapped, 43.3% was held by ion-exchange, 9.7% was complexed with functional groups, and 38.5% was accumulated inside cells. Using this approach, Yuan et al. (2017) reported that 8.55% of the adsorbed $Pb^{2+}$ was by physical entrapment, 35.74% by ion-exchange, 1.78% complexed with functional groups, and 53.93% accumulated inside cells. The large percentage of intracellular accumulation and small percentage of binding by functional groups are problematic. The kinetics of pyromorphite formation process is very rapid once both P and Pb become available (Chrysochoou et al., 2007), and Bai et al. (2014) noted the formation of $Pb_5(PO_4)_3OH$, $Pb_5(PO_4)_3Cl$, and $Pb_{10}(PO_4)_6(OH)_2$ on cell walls. Thus, EDTA is unlikely able to fully account for Pb-P precipitates on the cell surface. Biomass from phosphate accumulating organisms, such as *Ochrobactrum cicero*, *Stenotrophomonas maltophilia*, and *Pseudomonas putida*, has been used as an effective bio-sorbent to immobilize Cd, Cu, Pb, and Zn (Li et al., 2022). Biosorption was both pH and strain dependent with maximum immobilization (averaged 64%, 84%, 45%, and 64% of Cd, Cu, Pb, and Zn, respectively) occuring at pH ~6.

## 2.3 Practical considerations of PSB for Pb remediation

Concerns of using highly soluble phosphates or phosphoric acids for Pb remediation highlight the importance of using PSB along with soil amendment of poorly soluble P compounds such as rock phosphate. Existing laboratory research generally supports about 10–30% enhanced immobilization of Pb in contaminated soils upon inoculation of PSB (Table 2). For instance, Park et al. (2011b) reported that Pb immobilization in soil was enhanced by 13.7% and 26.4% using PSB strains, *Pantoea* sp. and *Enterobacter* sp., respectively, with phosphate rock amendment of 800 mg/kg. Li et al. (2022) reported 7.79% and 22.18% increase in the amount of Pb immobilized in a soil spiked with $Pb(NO_3)_2$ at 60 and 100 mg Pb/kg, respectively, due to inoculation with a PSB *Citrobacter farmeri* CFI-01. In most of the studies referenced in Table 2, Pb mobilization rate was found to be positively correlated with the amount of phosphate solubilized. Apparently, the efficiency of Pb immobilization varied with PSB, soil Pb levels, and the amount of P added into the soil. While these studies showed promising performance for Pb remediation, optimization of PSB is needed for site-specific conditions when applied in the field. The following two criteria are especially germane when selecting PSB for field applications: phosphate solubilization capacity and inhibition of PSB by Pb.

### 2.3.1 Phosphate solubilization capacity—

Numerous studies screened and isolated highly efficient PSB that can increase phosphate ions for immobilization of Pb (Table 1). Phosphate solubilization capacity is determined by inoculating PSB into a fixed volume of liquid medium (~100 mL) supplemented with an insoluble P source followed by measuring phosphate concentration in the medium during the incubation (typically for 7 d). Agar plates have also been used to screen PSB which produce acids to form a clear zone or halo around the colonies. Screening on agar medium is no longer considered a suitable test, and phosphate solubilization ability of microorganisms must be observed in liquid media. The insoluble P sources include $Ca_3(PO_4)_2$, powdered phosphate rock, $AlPO_4$, $FePO_4$, and lecithin (Table 1). Use of $Ca_3(PO_4)_2$ is perhaps the most popular method [e.g., the National Botanical Research Institute’s Phosphate (NBRIP) growth medium] with numerous reports of phosphate solubilization capacity in the range of 100–200 mg/L (Melo et al., 2018; Panhwar et al., 2014). Some PSB possessing extremely high $Ca_3(PO_4)_2$ measured phosphate solubilization capacity include *Pseudomonas trivialis BIHB 745* for 827 mg/L (Vyas and Gulati, 2009), *Serratia marcescens* RP8 for 974 mg/L (Misra et al., 2012) and *Acinetobacter sp.* ASL12 for 717 mg/L (Liu et al., 2014). The reported phosphate solubilization capacity measured with $FePO_4$ and $AlPO_4$ were much lower (5–50 mg/L), possibly due to their lower solubility than $Ca_3(PO_4)_2$ (Chen et al., 2014; Oliveira et al., 2009). In another study, Wan et al. (2020) took a stepwise acclimation approach using $Ca_3(PO_4)_2$, phytate, $FePO_4$, and $AlPO_4$ and identified a PSB (*Acinetobacter pittii* gp-1) that can solubilize multiple P sources. Apparently, targeted application of PSB depends on P speciation in the soil. Rock phosphate is an attractive amendment because it may supply phosphate gradually to the soil in aqueous phase for long-term Pb immobilization in the environment (Ma and Rao, 1999; Ma et al., 1995), but few studies examined PSB’s phosphate solubilizing capacity with rock phosphate (Park et al., 2011b).

**2.3.2 Minimum inhibitory concentration of Pb—**Environmental contamination by heavy metals generally induces morphological and physiological changes within microbial communities. Because Pb is bacteriostatic or bactericidal to many microorganisms, bacteria-induced immobilization of metals is unlikely to occur if heavy metals are present at concentrations that are toxic to the inoculated bacteria. PSB isolated from contaminated soils likely develop resistance mechanisms that enable them to survive and remain active in Pb contaminated environment. Therefore, isolating Pb resistant strains typically involves determination of the minimum inhibitory concentration (MIC), which is defined as the lowest concentration of Pb that inhibits visible growth of the isolate (Muñoz et al., 2012). The MIC value is obtained by incubating the PSB in varying concentrations of soluble Pb in the inoculated growth medium (typically at 28–30± C for 2–7 d) followed by growth measurements of the colony counts or optical density. Strains with high Pb resistance typically have a MIC greater than 1 mM (Teng et al., 2019). For instance, the MIC was found to be 0.6 mM for *Bacillus megaterium*, 2.5mM for *Pseudomonas marginalis* (Das et al., 2016; Roane, 1999), 1.21 mM for *Agrobacterium tumefaciens,* and 3.62 mM for *Acinetobacter* sp. (Zhang et al., 2011). It should be noted that visual observation of the colony growth can be subjective. Park et al. (2011b) evaluated MIC bacteria growth by measuring optical density at 600 nm wavelength, and they found a linear relationship between Pb concentration and absorbance. In this case, a MIC value cannot be readily obtained for the linearity observed. Several workers used the Maximal Tolerable Concentration (MTC), the highest Pb concentration that allows bacterial growth, as an alternative metric of Pb resistance (Yuan et al., 2017). The sensitivity of PSB to other environmental factors such as pH, temperature, and salinity can be tested in a similar fashion and will be important considerations for field applications, though bacteria have been reported to solubilize phosphate under abiotic stresses such as drought, low or high pH, salinity, and temperature (Chen and Liu, 2019).

## 3. Immobilization of Lead by Sulfate Reducing Microorganisms

Sulfate reducing microorganisms (SRM) have a major role in the cycling of sulfur in the environment (Leloup et al., 2009; Nakagawa et al., 2012; Vigneron et al., 2021; recently reviewed by Jørgensen et al., 2019). In aquatic systems, anoxic layers accumulate $H_2S$ and favor a reducing environment, thus allowing for the production of reduced divalent metals that bond to sulfide to form stable metal sulfides (Leloup et al., 2009; Nakagawa et al., 2012; Niu et al., 2018). Furthermore, the sulfur cycle is inextricably linked to the carbon cycle through remineralization organic matter carbon in sediment as evidenced by the inverse correlation of $H_2S$ concentration with organic matter (Holmkvist et al., 2011; Jørgensen, 1977; Westrich and Berner, 1984). SRM thrive in the sulfate reducing zone of sediments and can persist in the methanogenic zone where sulfate concentrations are low; scarce amounts of sulfate are likely produced by the reoxidation of sulfide in the presence of deeply buried oxidized iron in sediments (Holmkvist et al., 2011; Wehrmann et al., 2017). In addition, carbon also can be assimilated by anoxygenic phototroph oxidation of sulfides, thus completing the sulfur cycle (Nakagawa et al., 2012). Many SRM are resistant to heavy metals, can thrive in a harsh acidic or alkaline environment, withstand high salinity, and can use a variety of organic compounds as electron donors to reduce sulfate or sulfite to sulfide and sequester Pb or other metals as metal-sulfides (Cao et al., 2012; Martins et al., 2009; Utgikar et al., 2002; Vavourakis et al., 2019; recently reviewed by Ayangbenro et al., 2018). Thus, engineering applications harness the microbial processes to remediate Pb and other heavy metals from acid mine drainage (Elliott et al., 1998; Liu et al., 2017b; recently reviewed by Munyai et al., 2021); contaminated sediments (Li et al., 2017; Niu et al., 2018); wastewater (Kieu et al., 2015; Zhang et al., 2016a), and other environmental media (Beyenal and Lewandowski, 2004; Lin et al., 2010). This section focuses on Pb and toxic metal sequestration associated with sulfate reduction and molecular insights into these processes.

## 3.1 Biogeochemical processes of sulfate reduction and Pb immobilization

Sulfate reducing microorganisms (SRM) are ubiquitous in freshwater, brackish and marine environments (Jørgensen, 1977; Leloup et al., 2009; Li et al., 1999; Nakagawa et al., 2012; van Vliet et al., 2021; recently reviewed by Jørgensen et al., 2019; Wasmund et al., 2017) and have been isolated from diverse sources including dairy products, the human gastrointestinal tract, wastewater, sediment (Leloup et al., 2009), and low sulfate ground water (Bell et al., 2020; Feng et al., 2017; Doyle et al., 2018; Martins et al., 2009; Widdel and Pfennig, 1981). SRM live under anoxic conditions and use sulfate as a terminal electron acceptor to produce $H_2S$; the genes associated with these processes are found in anoxic waters. Sulfur cycling relies on both anoxic and oxic conditions where, in addition to sulfate reduction to sulfides by SRMs, chemoautotrophic or photoautotropic sulfur bacteria or abiotic processes oxidize sulfides to sulfates (Fortin et al., 1995).

In aqueous environments, such as lakes and marine systems, pore water and leachates, SRM reduce sulfate in the presence of organic matter, and through a sulfite intermediate, produce hydrogen sulfide ($S^{2-}$) and bicarbonate, summarized in the following reactions (Neculita et al., 2007):

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S\,.$$

Sulfide ions can complex with divalent metals, such as $Pb^{2+}$, to form an insoluble precipitate (Fortin et al., 1995):

$$H_2S + M^{2+} \rightarrow MS + 2H^+,\ \text{where}\ \ M = \ \text{metal}\,.$$

In the presence of $Pb^{2+}$, lead sulfide is formed:

$$H_2S + Pb^{2+} \rightarrow PbS + 2H^+$$

Electron microprobe analysis of marsh sediments revealed that lead sulfide also can aggregate with $FeS_2$ and be sequestered as $FePbS_2$ in an anoxic environment where ferrous iron ($Fe^{2+}$) is more reactive than $Pb^{2+}$ and $FeS_2$ is formed preferentially (Moreau et al., 2013). Similarly, As and Cu may be sequestered as $FeAsS_2$ and $FeCuS_2$ whereas the sulfide of Cd may prefer to precipitate with Zn.

The above processes can be limited by the ambient environment, especially carbon sources. In a two-year Danish fjord study, sulfate reduction was coupled with the availability of organic matter and only a fraction (10%) of the produced sulfide formed metal-sulfide precipitates (Jørgensen, 1977). In a monomictic lake, the SRM *Algidimarina propionica* (Desulfobacteraceae) was correlated with sulfate reduction, a process that was limited by sulfate concentration as well as dissolved oxygen, temperature, pH, and availability of organic matter (Nakagawa et al., 2012; Sánchez-Andrea et al., 2014).

## 3.2 Functional genes associated with sulfide-mediated heavy metal sequestration

**3.2.1 Sulfate and sulfite reduction functional genes and microbes—**Gene products for sulfate and sulfite reduction pathways have been well described and genomic analysis provides insights into the reductive metabolism in an anoxic environment (Anantharaman et al., 2018; Vigneron et al., 2021). Of these, dissimilatory sulfate and sulfite reduction are most prevalent in Pb immobilization (Fig. 3). Sulfide is generated by sulfate adenylyltransferase (*sat*), adenylyl sulfate reductase alpha and beta subunits (*aprAB*) and which complexes with a quinone-interacting membrane-bound oxidoreductase (*qmoABC*) complex to transport electrons, and dissimilatory sulfite reductase alpha and beta subunits (*dsrAB*) (Friedrich, 2002; Fritz et al., 2000; Müller et al., 2015; Ramos et al., 2012; Wagner et al., 1998; Wasmund et al., 2016; Zane Grant et al., 2010). The *dsr* operon is comprised of *dsrAB* (catalytic core), *dsrC* (substrate delivery), *dsrT* (putative regulation), *dsrEFH* (sulfur carrier), *dsrMKJOP* (transmembrane complex involved in electron transport; Anantharaman et al., 2018; Ramos et al., 2012; van Vliet et al., 2021; Wasmund et al., 2016); a subset of these gene products are involved in the reverse reaction, i.e., oxidation of sulfide to sulfite and sulfate. For example, microbial oxidation of sulfide (electron donor) can involve the *dsrAB* and *dsrEFH* gene products. While not definitive, presence of *dsrD* may be indicative of preference for the reduction pathway (Anantharaman et al., 2018; Bell et al., 2020; Umezawa et al., 2020). The *dsrD* gene product has been shown to be down regulated in a high sulfide (10 mM) environment, possibly as a feedback mechanism to preclude toxic levels of sulfide (Caffrey and Voordouw, 2010). In the presence of Pb, less soluable sulfide precipitates can form in anoxic environments and sequester Pb.

The highly described Gram-negative dissimilatory sulfate reducing mesophilic bacteria, which belong to class Deltaproteobacteria, families Desulfovibrionaceae, Desulfobacteraceae, Desulfohalobiaceae, Desulfonatronumaceae, Desulfomicrobiaceae, and Syntrophobacteraceae include genera such as *Desulfovibrio*, *Desulfomicrobiium*, *Desulfobulbus*, and *Syntrophobacter* (Bao et al., 2021; Leloup et al., 2009; Müller et al., 2015; van Vliet et al., 2021). Other lessor represented sulfate reducers include members from the phyla Caldiserica (*Caldiserica* sp.), Firmicutes (*Thermanaeromonas toyohensis*, *Desulfotomaculum* sp.), Actinobacteria (*Gordonibacter pamelaeae* and *Thermodesulfobium narugense*), Thermodesulfobacteria (*Thermodesulfobacterium commune*), Nitrospirae (*Thermodesulfovibrio yellowstonii*, and *Thermodesulfovibrio islandicus*), Thaumarchaeota (*Aigarchaeota archaeon*), and Euryarchaeota (*Archaeoglobus fulgidus*, *Archaeoglobus profundus*) based in part on the presence of dissimilatory sulfate reductase genes *dsrAB*. Families Desulfobulbaceae and Desulfomicrobiaceae were positively correlated with reduced sulfur species (Bao et al., 2021; Leloup et al., 2009).

Communities in the anoxic zone are not as well described as ones from more oxic zones, however 16S rRNA and sulfate/sulfite reduction related gene sequencing has revealed new SRMs that harbor genes for dissimilatory sulfate (*dsrAB*) and/or anaerobic sulfite reduction (*asrAB*), such as members of the Class Dehalococcoidia (Phylum Chloroflexi), Planctomycetes, *Candidatus* Rokubacteria, *Candidatus* Woesearchaeota, *Candidatus* Omnitrophica, and *Candidatus* Parcubacteria *(Candidatus* Nealsonbacteria*, Ca.* Zixibacteria *and Ca.* Abyssubacteria; Hug et al., 2016; Vavourakis et al., 2019; Vigneron et al., 2020; Wasmund et al., 2016). While the sulfate reducing genes (e.g. *sat*, *apr* and *dsr*) may be detected, their presence does not confirm sulfate reduction activity nor does it rule out the ability to undergo the reverse dissimilatory oxidation of reduced species, such as sulfide (Umezawa et al., 2020).

Several facultative anaerobes harbor alternative dissimilatory sulfite reductase genes (Figure 3). The *sirA* (*Shewanella oneidensis*) and *mccA* (*Wolinella succinogenes*) gene products, cytochrome *c* sulfite reductases, also reduce sulfite to sulfide and are generally found in microorganisms that cannot respire sulfate (Hermann et al., 2015; Kern et al., 2011; Sanchez-Andrea et al., 2011; Shirodkar et al., 2011). *S. oneidensis* was demonstrated to reduce metals using sulfite as an electron acceptor, generating ZnS (Xiao et al., 2015), CuS (Zhou et al., 2016), FeS (Lutterbach et al., 2009), and $Ag_2S$ (Voeikova et al., 2016). While the literature is sparse on *W. succinogenes* metal resistance, the organism tolerated 20 mg $L^{-1}$ Cd but not Zn or Cu at 30 mg $L^{-1}$ or 2 mg $L^{-1}$, respectively (Sinbuathong et al., 2013). Both *S. oneidensis* and *W. succinogenes* formed a black precipitate on Pb acetate test strips *in vitro* which is indicative of $H_2S$ production and PbS formation (Tanner et al., 1981; Wu et al., 2015), suggesting the potential for Pb resistant strains to generate PbS.

Sulfite reduction also is catalyzed by the *asrAB* gene product, siroheme-dependent anaerobic sulfite reductase (Fig. 3), described in Gammaproteobacteria (*Salmonella* spp. and *Edwardsiella* spp.), Firmicutes, Spirochaetes, and Fusobacteria, as well as newly identified *Candidatus* Omnitrophica, *Candidatus* Planctomycetes, *Candidatus* Riflebacteria, and *Candidatus* Parcubacteria (Anantharaman et al., 2018; Huang and Barrett, 1991; Vigneron et al., 2020). Strictly anaerobic sulfite reductase differs from its assimilatory cysteine synthesis analog, as described in studies on *S. typhimurium* (Haber et al., 1980; Huang and Barrett, 1990; Huang and Barrett, 1991). In assimilatory sulfate reduction, cysteine regulates $H_2S$ production with little to no accumulation. However, *asrAB* sulfite reduction in *Salmonella* spp. is an anaerobic dissimilatory process and $H_2S$ accumulates; $H_2S$ is not dependent on cysteine biosynthetic pathway regulation and gene expression (Hallenbeck et al., 1989; Huang and Barrett, 1990). Because genes for sulfate reduction were absent from *Candidatus* Riflebacteria and *Candidatus* Omnitrophica (Anantharaman et al., 2018), it is likely that sulfite reduction is a strictly anaerobic process similar to that in *S. typhimurium* where $H_2S$ accumulates and if Pb tolerant, PbS produced.

#### 3.2.2 Use of sulfite reduction genes as environmental genetic markers—

The dissimilatory sulfite reductase genes, *dsrAB*, *dsrA*, and *dsrM*, have been used as genetic markers to denote sulfate reduction in environmental systems (Ben-Dov et al., 2007; Müller et al., 2015; Nguyen et al., 2017; Scholten et al., 2005). Quantitative and qualitative enumeration of SRMs in freshwater and marine systems, as well as industrial wastewater, have been done with the adenylyl-sulfate reductase subunit alpha gene (*aprA*) usually in combination with the *dsrA* or *dsrM* genes (Ben-Dov et al., 2007; Keshri et al., 2015; Scholten et al., 2005). Deltaproteobacteria harboring *sat*, *aprAB*, *dsrAB*, and *dsrD* (dissimilatory sulfite reductase D) genes was unexpectedly found in low sulfate groundwater, which suggests, but not confirms, metabolic activity (Bell et al., 2020).

The *dsrA* and *dsrM* genes are present in marine sediments in both the sulfate reducing (20–150 cm) and deeper methanogenic (280–440 cm) zones, with no loss of bacterial community richness throughout the zones, to include the intervening transition zone (Leloup et al., 2009). In the sulfate reducing zone, the *dsrA* gene was associated with dominant families Desulfobacteraceae and Desulfobulbaceae*, Desulfobacterium anilini*, which was present in the transition and methane-rich zones, and Firmicutes, in the methane-rich zone. Dissimilatory-type sulfite reductase genes (*dsrAB*) have been detected in extreme environments including thermal water and sediment from heavy metal mining operations (Bao et al., 2021). Using polymerase chain reaction amplification and 16S rRNA gene analysis, hydrothermal water from a Cu-Pb-Zn mine was shown to contain the *dsrAB* genes which were attributed to *Desulfotomaculum* spp., *Desulfovibrio* spp., *Desulfococcus* spp., *Thermodesulforhabdus norvegica*, and *Thermodesulfobacterium* spp., among others (Nakagawa et al., 2012). The *dsrB* gene was detected in an acid mine drainage contaminated river (Hengshi River, China); classes *Clostridia* and Deltaproterobacteria and families Syntrophobacteraceae and Desulfobacteraceae were dominant in sediment (Bao et al., 2021).

*Desulfovibrio spp*. and *Desulfotomaculum spp*. have been isolated from Kidd Creek Cu-Zn sulphide mine tailings (Fortin et al., 1995). Tailings from the mine contained pyrite, pyrrhotite, sphalerite, chalcopyrite and galena; metals including Zn, Pb, Cd, and Cu, were released into the associated pore water as part of the refinery process (Al et al., 1994). The SRMs reduced sulfate to hydrogen sulfide which, upon dissolution, caused the formation of metal-sulfide precipitates when present (Al et al., 1994; Fortin et al., 1995). Dissimilatory-type sulfite reductase subunit β (*dsrM*) gene also was detected in metal contaminated sediments (Fe, Mn, V, Cr, Ni, Cu, Zn, Sr, Cd, Pb) and was more prevalent in the anaerobic rhizosphere of the marsh plant, *Scirpus triqueter*, than in surrounding sediment, suggesting a presence of SRM (Niu et al., 2018). Even though SRM abundance and $SO_4^{2-}$ concentration and Pb and sulfide concentration were positively correlated, nanoparticles observed throughout the rhizosphere sediments only contained S and Fe, Ag-Ca, or Pt-Cu.

### 3.3 Lead and toxic metal remediation by sulfate reduction sequestration

Leveraging the sulfur cycle and adapting bacterial sulfate reduction to alleviate anthropogenic derived metal contamination has been explored by many researchers (Fortin et al., 1995; Foucher et al., 2001; Liu et al., 2017a; Nguyen et al., 2017; Yin et al., 2020a; Yin et al., 2020b; recently reviewed by Ayangbenro et al., 2018). SRM biogenic sulfide production may be a less expensive and more environmentally friendly approach for Pb sequestration as PbS (Tong et al., 2021; Table 3). Generally, through a plethora of engineering approaches designed for specific matrices, the microorganisms and their metabolism are optimized to maximize precipitation of PbS. Formation of these insoluble metal-sulfide precipitates are detected by X-ray diffraction analysis (XRD), scanning electron microscopy (SEM), and various spectroscopic analytical approaches such as energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), fourier transform infrared spectroscopy (FTRI), induced coupled plasma optical emission spectroscopy or mass spectroscopy (ICP-OES/MS), atomic absorption spectroscopy (AAS), and synchrotron-based x-ray diffraction spectroscopy (SXRD) or synchrotron-based X-ray absorption fine structure spectroscopy (XAFS; Beyenal and Lewandowski, 2004; Karna et al., 2016; Li et al., 2016b; Nguyen et al., 2017; Pérez-López et al., 2018; Sinharoy and Pakshirajan, 2019).

**3.3.1 SRM-assisted biofilm reactor—**The use of sulfate reducing biofilms is an effective approach for precipitating Pb. *Desulfovibrio desulfuricans* biofilms grown anaerobically on both quartz ($SiO_2$) and hematite ($Fe_2O_3$) effectively immobilized Pb (Beyenal and Lewandowski, 2004). Less $H_2S$ production and Pb immobilization occurred on the hematite-grown biofilm, probably due to elevated concentrations of $Fe^{2+}$ in the system competing for the scarcer sulfide. In an inversed fluidized bed reactor, Pb precipitation and recovery from synthetic wastewater, which contained 50 mg/L iron sulfate, was influenced by sulfide concentration (Villa-Gomez et al., 2011). Lead sulfide precipitates were suspended in the liquid phase in the higher sulfide concentration (648 mg/L) reactor whereas Pb precipitates were associated with the biomass at lower sulfide (44 mg/L) and thus, harder to recover, even though both concentrations had similar Pb recovery (58% versus 60%). In the presence of micro-sized $Fe^0$ particles designed to scavenge available $O_2$, *Desulfobulbus propionicus* and *Desulfovibrio vulgaris* were able to immobilize sediment Pb (380.6 mg $kg^{-1}$), Cu (174.8 mg/kg), Cd (94.4 mg/kg), and Zn (1311.3 mg/kg) by 90.69% to 100%, depending on the metal (Li et al., 2016b). These authors suggested that the heavy metals combined with reduced sulfate metabolites to form insoluble compounds.

Nguyen et al. (2017) used a continuous moving bed biofilm reactor, populated with *Desulfomicrobium escambiense*, *Desulfovibrio carbinolicusto*, *Desulfobacterium autotrophicum*, *Desulfomicrobium salsugmis*, and *Desulfovibrio vulgaris* to determine optimal SRM communities and Pb concentrations to maximize removal rate of Pb from the wastewater system. Following *dsrM* gene-based denaturing gradient gel electrophoresis, fluorescent in situ hybridization, and chemical analysis, it was determined that *Desulfomicrobium escambiense* and *Desulfovibrio carbinolicusto* were instrumental in Pb removal and tolerated up to 40 mg $L^{-1}$ $day^{-1}$ for 20 days, however populations decreased by 40 days, contributing to reduced Pb removal compared to 20 mg $L^{-1}$ $day^{-1}$ and 30 mg $L^{-1}$ $day^{-1}$ removal was observed (91% versus 99%–100%). *Desulfobacterium autotrophicum* and *Desulfomicrobium salsugmis* tolerated up to 20 mg $L^{-1}day^{-1}$ for 40 days however *Desulfovibrio vulgaris* was inhibited by Pb concentrations used in the study. Lead precipitates attached to the cells were observed by scanning electron microscopy. Hoa et al. (2007) described a two-phase up flow anaerobic sludge blanket reactor system for Pb removal. In the first phase, SRM converted sulfate to sulfide in the absence of Pb, and in the second phase, Pb ($Pb[NO_3]_2$; 50 mg/L) was added to the sulfide-rich phase and 85–95% of the Pb was precipitated to lead sulfide and then removed from the system. The stoichiometric concentrations of $Pb^{+2}$ and $SH^-$ were maintained to maximize PbS precipitation. Using highly controlled systems, i.e., optimizing sulfate, sulfide, Pb, and carbon source, as well as pH and temperature, or through less engineered approaches, Pb can effectively be precipitated as a sulfide and removed from the environment.

#### 3.3.2 Applications with acid mine drainage—

Both abiotic and biotic processes have been used to remediate acid mine drainage (AMD), which is typically contaminated with a variety of toxic metals (see more in-depth reviews on specific related topics by Papirio et al., 2013; Rambabu et al., 2020; Simate and Ndlovu, 2014; Tong et al., 2021). Although chemical processes are effective, some create a toxic environmental footprint that requires additional remediation (Tong et al., 2021). For the biotic processes, finding SRM that can survive in the extreme pH range (2–4) and are resistant to the toxic metals is challenging. Several configurations that enrich for SRM and the production of sulfide in the presence of toxic metals have been demonstrated (Fig. 4): 1) separate biogenic $H_2S$ production and toxic metal precipitation phases (Foucher et al., 2001); 2) inoculate the system with SRM (e.g. activated sludge) and demonstrate sulfate removal or $H_2S$ production prior to introducing AMD with toxic metals (Villa-Gomez et al., 2011); 3) increase the pH of the solution with lime or other alkaline material and then inoculate with SRM with or without the toxic metals (Sato et al., 2022; Sato et al., 2019); 4) inoculate the system with SRM and sequentially lower pH and then add AMD (acid/toxic metals; Elliott et al., 1998); 5) select acid and/or toxic metal tolerant SRM (e.g. mining site sediment, battery industry wastewater; enrichment and isolated cultures) and add to the system (Dev et al., 2021; Ňancucheo and Johnson, 2012; Nguyen et al., 2017; Zhang et al., 2016b); or 6) inoculate the system with a SRM source (e.g., mud from under coal gangue pile, pond sediment, activated sludge, isolated cultures) and concurrently introduce aqueous toxic metals (Chen et al., 2021; Liu et al., 2017b; Makhathini et al., 2021; Wang et al., 2021; Zhang et al., 2016a). Optimized systems have been demonstrated to remove greater than 99.5% Pb (loading rate 9.2 g/m$^3$-d Pb(II)) and Hg (loading rate 2.6 g/m$^3$-d Hg(II)) as PbS and HgS (Zhang et al., 2016a).

Identifying economical nitrogen and carbon sources also are required to make using SRM advantageous. In one study, a packed bed reactor with introduced ground marine waste (dead crustaceans and mollusks and fish scales) as an organic nitrogen source was used to select for sulfate reducers and remove metals from acid mine waste (Dev et al., 2016). The marine waste performed better than tryptone, yeast extract, corn steep liquor, ammonium bicarbonate ($NH_4HCO_3$) and ammonium chloride ($NH_4Cl$) and when introduced into the reactor; 62–66% of the sulfate and 66–75% of the divalent metals (Fe, Cu, Zn, Ni, Mg) were removed. The marine waste selected for sulfate reducers which accounted for 87–89% of the bacterial community and the genera *Desulfovibrio* (Deltaproteobacteria), *Desulfotomaculum* (Firmicutes), *Desulfobacter* (Deltaproteobacteria), classes Clostridia and Synergistia (Synergistetes), and members of the phylum Bacteroidetes dominated (Dev et al., 2016). Other engineered systems designed to remove toxic metals, which may be transferable to Pb remediation, have experimented with less costly carbon sources, such as rice husk (Sato et al., 2022; Sato et al., 2019), rice bran (Sato et al., 2022), sugar cane slag (Wang et al., 2021), corn cob (Wang et al., 2021), sunflower straw (Wang et al., 2021), walnut shells (Chen et al., 2021), and anaerobic sludge (Makhathini et al., 2021). In one study with three different reactor scenarios using vertical flow sulfate reducing bioreactors, AMD was neutralized with limestone, rice bran served as a carbon source, and the system was inoculated with soil as a source for SRM and Fe was removed from the system as applicable (Sato et al., 2019). After approximately four to five months, *Desulfosporosinus meridiei* dominated and other *Desulfosporosinus* (*D. fructosivorans*), SRM-like *Clostridium*, *Ruminococcus*, *Desulfovibrio* and *Desulfobulbus* were prevalent in each system. In a previous study using a similar system, Zn (83–100%), Cd (93–100%), and Cu (99–100%) were removed and *Desulfatirhabdium butyrativorans* was dominant (Sato et al., 2018). Sulfate reduction was correlated with metal recovery rate. In another system using dynamic columns inoculated with SRM to evaluate the efficacy of different carbon sources, iron scrap was added to enhance the system performance (Wang et al., 2021). All three carbon sources (sugarcane slag, corn cob, sunflower straw) supported efficient removal of $Cr^{6+}$ (95–97%); the corn cob carbon source yielded maximum $Cr^{3+}$ (86%) and $SO_4^{2-}$ (75%) removal.

Passive systems, which may be more economical and easier to implement at the source, also can be effective at removing Pb from mine waste. In an aqueous laboratory scale open tank system, SRM and iron reducing bacteria were added to sulfidic mine waste (chalcopyrite tailings) and incubated for 60 days, resulting in decreased dissolved metals (Fe, Cu, Zn), including an 85% reduction in dissolved Pb (Liu et al., 2017b). At the end of the incubation period, *Desulfosporosinus medirie* (Firmicutes) was the dominant sulfate reducer. Seven months after the SRM and iron-reducing bacteria were applied to Pb-Zn sulfide mine tailings in the field, Fe, As, and Sb, as well as S, were reduced however Pb, Zn, and Cd were indistinguishable from initial concentrations. However, toxicity was reduced as evidenced by moss and plant growth over the initially barren area.

In an effort to assess efficiency of constructed wetlands to remove metals, microcosm tanks containing walnut shells as a carbon source were inoculated with pond sediment and activated sludge and seedlings (*Iris pseudoacorus* L.) were planted in the microcosm (Chen et al., 2021). The synthetic AMD (pH 4), containing $FeSO_4$ (50 mg $L^{-1}$), and $K_2Cr_2O_7$, $CuCl_2$, $CdCl_2$, and $ZnSO_4$ (concentration at 5 mg $L^{-1}$ for each) was introduced into the microcosm which was reinoculated 30 days later. Sequential “fill and drain” batch operations occurred over a period of 200 days with several nutrient and carbon source deviations. Over 50% of Cu was sequestered as copper sulfide; lower concentrations of other metal sulfides were identified, primarily in the walnut shell substrate. Few SRM were detected in Phase I when acidic and oxygenic conditions prevailed; *Desulforegula* and *Desulfovibrio* were detectable at low numbers. However, *Desulfobulbus, Desulfosporosinus,* and *Desulfatirhabdium* were selected for in the control and treated constructed wetland microcosms following inoculation of both with wastewater. Batch bioreactors supplemented with various carbon sources and artificial AMD, successfully removed up to 100% of Al (61 mg/L), Fe (171 mg/L), and Mn (2 mg/L), presumably as metal sulfides (Neculita et al., 2011). During the 35-day period, elevated SRM counts positively correlated with sulfide production and increase in pH; a 1:1 sawdust and cow manure mixture was most effective at pH 3. While not all studies report results in terms of Pb removal, the processes involved are applicable because formation of metal sulfides from toxic divalent metals is demonstrated as the removal mechanism. Furthermore, metal resistance genes have been shown to be co-located, thus SRM Pb tolerance is plausible (Kang and So, 2016; Leedjarv et al., 2008; Pal et al., 2017).

## 4. Immobilization of Lead through Microbial Induced Carbonate Precipitation

Microbial induced carbonate precipitation (MICP) is well documented and has been shown to be facilitated by Bacteria, Archaea, and Eukaryotes under favorable conditions, i.e., an alkaline environment, presence of a nucleation site, and adequate concentration of substrates. The process has shown promise for soil and sand stabilization (Graddy et al., 2021; Montoya et al., 2014; Ohan et al., 2020), stone and cement repair (Pei et al., 2013; Su et al., 2021), and toxic metal remediation (Liu et al., 2021; Xue et al., 2022; Zeng et al., 2021). MICP, which has been reported to modulate $Ca^{2+}$ levels intracellularly and extracellularly to optimize conditions for growth, is connected to carbon, nitrogen, and sulfur metabolism (Hammes and Verstraete, 2002; Schultze-Lam et al., 1996; Wright, 1999; recently reviewed by Castro-Alonso et al., 2019; Görgen et al., 2021). While most inorganic carbon generating mechanisms under alkaline conditions can produce calcium carbonate, potential engineering applications have selected for strains with high urease activity which results in higher yields of calcium carbonate from urea and maximizes performance for the intended purpose such as Pb immobilization (Table 4). Various polymorphisms, i.e., calcite, aragonite, vaterite and dolomite can accumulate, a process governed by species and/or biomineralization mechanism (Ferrer et al., 1988; Roberts et al., 2004; Zhang et al., 2019a). This section focuses on lead and toxic metal sequestration associated with MICP.

### 4.1 Biogenesis of carbonate for Pb immobilization

The most prevalent biotic scenarios with MICP involve ureolytic organisms, which have urease enzymatic activity that can convert urea to ammonium ($NH_4^+$) and carbonate ($CO_3^{2-}$) accompanied with increase in pH (Fig. 5). In turn, divalent cations, such as $Pb^{2+}$ and $Ca^{2+}$ can bind to carbonate and form precipitates, lead carbonate ($PbCO_3$) and calcium carbonate ($CaCO_3$). These processes are well tested and verified in the laboratory setting. Both active and passive biogenesis mechanisms have been described (Ferrer et al., 1988; Hammes et al., 2003; Roberts et al., 2004; Zhang et al., 2019a). Highest yields of microbial mediated $PbCO_3$ and $CaCO_3$ precipitates occur when urea is hydrolyzed by urease creating an alkaline environment through the reaction below as described by (Schultze-Lam et al., 1996) and adapted from (Stocks-Fischer et al., 1999).

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+$$

$$2NH_3 + 2H_2O \rightleftharpoons 2NH_4^+ + 2OH^-$$

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \rightleftharpoons CO_3^{2-} + 2NH_4^+ + 2H_2O$$

$$Cell^2 + Ca^{2+} \rightarrow Cell - Ca^{2+}$$

$$Cell{-}Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3$$

In the presence of $Pb^{2+}$ (and other divalent metals):

$$\text{Cell-Pb}^{2+} + CO_3^{2-} \rightarrow \text{Cell-PbCO}_3$$

In addition to urea hydrolysis (Graddy et al., 2021; Zhu et al., 2019b), other mechanisms generating or utilizing inorganic carbon have been described for MICP such as dissimilatory sulfate reduction (Lin et al., 2018; Wright, 1999), nitrification/denitrification (Eltarahony et al., 2020; Erşan et al., 2016; Zhu et al., 2019b), iron reduction (Kenward et al., 2009), methanogenesis (Kenward et al., 2009; Roberts et al., 2004; Su and Yang, 2021), fatty acid metabolism (Barabesi et al., 2007) and photosynthesis (Benzerara et al., 2014). Urease activity has been correlated with the *ureC* gene (Urease subunit alpha) in mining site isolates *Sporosarcina luteola* UB-3 and UB-5, *Sporosarcina soli* B-22, *Sporosarcina pastuerii* WJ-2, *Viridibacillus arenosi* B-21, *Enterobacter cloacae* Kj46 and KJ-47, and *Lysinibacillus sphaericus* KJ-64 (Cuaxinque-Flores et al., 2020; Kang and So, 2016). Carbonic anhydrase, which catalyzes reversable hydration of $CO_2$ forming $HCO_3^-$, also has been implicated in calcite formation in *Bacillus* sp. AP4, *Bacillus megaterium* AP6, and *Bacillus simplex* AP-9 (Achal and Pan, 2011). In *Bacillus subtillis* PB-19, targeted mutations in the *lcfA* operon (*lcfA*, *ysiA*[*fadR*], *ysiB* [*fadB*], etfB, and *etfA*), which is linked to fatty acid metabolism impaired $CaCO_3$ formation indirectly suggesting a role of this operon in formation of the $Pb^{+2}$- $Ca^{2+}$ carbonate matrix (Barabesi et al., 2007; Frandi et al., 2011; Fujihashi et al., 2014).

Mechanisms linked to carbonate production do not occur in isolation. Isolates from Pb, As, and Zn contaminated mine tailing soil identified as *Bacillus* sp., linked a reduction in exchangeable Pb and increase in Pb carbonate to urease, dehydrogenase and phosphatase activities, suggesting multiple mechanisms involved in Pb immobilization (Govarthanan et al., 2013). Dehydrogenase activity, which is involved in organic compound oxidation-reduction reactions, provided insight into metabolic status under the $PbCO_3$ synthesis conditions. *Kocuria flava*, a Pb resistant isolate from a Pb mining area, also formed Pb carbonate, which positively correlated to urease and dehydrogenase activities and negatively correlated to toxicity (Achal et al., 2012).

### 4.2 Mechanism of Pb immobilization through carbonate biomineralization

Precipitating Pb in its carbonate form has been described extensively as a potential Pb remediation strategy with both biotic and abiotic processes involved in Pb carbonate synthesis. While this section focuses on the biotic process, one cannot ignore the abiotic mechanisms because both can co-occur with ion exchange of $Pb^{2+}$ for $Ca^{2+}$. Because heavy metals of similar ionic radius to $Ca^{2+}$, such as $Pb^{2+}$, can co-precipitate with and/or substitute for $Ca^{2+}$, this principal can be applied to Pb remediation. Both natural limestone formations and limestone enriched soil and water field applications can sequester Pb as minerals e.g., $PbCO_3$, $Pb_3 (CO_3)_2(OH)_2$, and PbO (Aziz et al., 2008; Berger et al., 2000; Caraballo et al., 2009; Fuchida et al., 2020). In addition, Pb, Cd, Cu, and Zn can bind to calcite surfaces such as aragonite and calcite mollusk shells (Chada et al., 2005; Du et al., 2011; Elzinga et al., 2006). Calcite crystals have been reported to preferentially incorporate metals ($Cu^{2+}$, $Zn^{2+}$, $Cr^{4+}$) during formation and $Pb^{2+}$ has a high affinity to calcite, strongly sorbing to the surface (Chada et al., 2005; Elzinga and Reeder, 2002; Elzinga et al., 2006; Mugwar and Harbottle, 2016; Rouff et al., 2006; Tang et al., 2007). Because the bacterial cell wall (Kulczycki et al., 2002; Pei et al., 2013; Perito et al., 2014) and associated exopolysaccharide or capsular polysaccharides (Azulay et al., 2018; De Philippis et al., 2011; Ercole et al., 2007; Su et al., 2021; Yin et al., 2020b) are electronegatively charged, they can serve as nucleation sites for calcium carbonate crystals in the presence of $Ca^{2+}$, inorganic carbon ($CO_2$, $CO_3{}^{2-}$,$HCO_3{}^{-}$), and alkaline pH (Hammes and Verstraete, 2002; Schultze-Lam et al., 1996). The same is true for biomineralization of cerussite in the presence of $Pb^{2+}$ (Fig. 5). Thus, biotic and abiotic precipitation as well as biosorption contribute to the sequestration of Pb. The associated mineral products are characterized by X-ray diffraction (XRD) patterns and scanning electron microscopy (SEM) images or through other modern analytical approaches.

Biomineralization of lead carbonate through MICP has been observed with a variety of species. *Sporosarcina globispora*, *Sporosarcina koreensis*, *Sporosarcina* sp., *Sporosarcina pasteurii*, *Bacillus lentus*, and *Terrabacter tumescens* incubation with $PbCl_2$ resulted in 100% removal of Pb, observed by SEM and XRD as extracellularly-bound Pb crystalline carbonate structures at pH 8–9 (Li et al., 2013; Li et al., 2016a). Greater than 97% Pb removal was observed for *Micrococcus* sp. and *Pararhodobacter* sp. resulting from co-precipitation of $PbCO_3$ and $CaCO_3$ (Gomaa, 2019; Mwandira et al., 2017). Both strains formed $PbCO_3$ biotically through the MICP mechanism and abiotically, through $Pb^{2+}$ substitution for $Ca^{2+}$ in the calcite crystalline structure. Urease positive *Raoultella planticola* VIP and the fungus *Metschnikowia pulcherrima* 29A were resistant to 700 mg/L $Pb(CH_3COO)_2$, precipitating it as $CaPbO_3$, lead oxide, and $PbCO_3$ cerussite (Eltarahony et al., 2021). *Penicillium chrysogenum*, isolated from Cr and Pb contaminated cement sludge, removed 99% of the Pb (Qian et al., 2017). Fungal uptake, through both biosorption and MICP mechanisms, accounted for Pb immobilization as 1–10 μm particles, visualized by SEM. Carbonate products included calcite, vaterite, hydrocerussite [lead hydroxycarbonate; $Pb_3(CO_3)_2(OH)_2$], and lead carbonate ($PbCO_3$); both $Ca^{2+}$ and $Pb^{2+}$ containing precipitates occurred in parallel. In another study, a Pb resistant ureolytic *Staphylococcus epidermis* strain (HJ2), isolated from heavy metal contaminated soil, similarly co-precipitated calcite and Pb carbonate, immobilizing 86% of the Pb present (He et al., 2019). In a yeast extract enriched medium, ureolytic *Sporosarcina pasteurii* removed almost 100% Pb in solution

(Jiang et al., 2019). Geochemical modelling revealed that >85% Pb was removed from the system resulting in co-precipitation of $PbCO_3$ and $CaCO_3$, with Pb carbonate intermediates, $(PbCl)_2CO_3$ and $Pb_3(CO_3)_2(OH)_2$.

### 4.3 Microbial Isolates for removal of Lead and toxic metals as carbonate precipitates

When microbial induced carbonate precipitation (MICP) is the method of choice to explore environmental Pb remediation options, urease producing Pb resistant microbial strains or consortia usually are selected and their efficacy for $PbCO_3$ biogenesis determined experimentally. Microbial strains recovered from toxic metal contaminated sites, e.g., mining soils and leachates, cements, and electronic waste, are good candidates for Pb remediation through the MICP mechanism (Table 4). Represented bacterial phyla include Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria and eukaryotes, Ascomycota and Ciliophora. Clearly, production of $PbCO_3$ has the potential to improve the overall health of the ecosystem by immobilizing Pb and alleviating Pb induced stress. Selecting for strains and consortiums that produce high urease activity and are resistant to toxic metals show much promise for environmental applications.

**4.3.1 Single isolates—**Ureolytic isolates recovered from environmentally contaminated sites also have been studied for their ability to precipitate metals. In addition to strains mentioned in Section 4.2, *Sporosarcina luteola* UB3 and UB5 were isolated from mine tailing contaminated with As, Pb, Zn, Cd, Cu, and Fe; the isolates were resistance to 25–50 mM $As^{5+}$, $Cr^{6+}$, $Fe^{3+}$, $Fe^{2+}$, $Co^{2+}$, $Rb^{+}$ $Sb^{3+}$; 5–10mM: $Mn^{2+}$, $As^{3+}$, $Cu^{2+}$, $Ni^{2+}$, $Ba^{2+}$; and <1mM: $Pb^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Hg^{2+}$, $Te^{4+}$ $Ag^{+}$ (Cuaxinque-Flores et al., 2020). Urease activity was correlated to metal resistance and precipitates included cerussite, calcite, and vaterite. In other studies, *Enterobacter cloacae*, *Viridibacillus arenosi*, *Sporosarcina soli*, *Lysinibacillus sphaericus*, and *Sporosacina pasteurii* were isolated from soil recovered from an abandoned mine (Kang et al., 2015; Kang and So, 2016). All strains harbored the *ureC* gene and had urease activity, were resistant to a variety of heavy metals and antibiotics and produced calcite. Two *Enterobacter cloacae* isolates produced a $PbCO_3$ precipitate which positively correlated with viability, thus immobilizing 68% of the Pb (Kang et al., 2015).

Bacterial isolates with no apparent previous exposure to heavy metals can mineralize Pb and other metals into carbonate compounds (Bai et al., 2021; Gomaa, 2019; Jiang et al., 2019; Kim et al., 2021; Li et al., 2013; Li et al., 2016a; Liu et al., 2021; Mugwar and Harbottle, 2016) (Xue et al., 2022; Zeng et al., 2021). Soil isolates, *Sporosarcina globispora*, *Sporosarcina koreensis*, *Sporosarcina pasterii*, *Sporosarcina* sp., *Bacillus lentus*, and *Pararhodobacter* sp., and well as culture collection isolates, *Terrabacter tumescens*, *Sporosarcina pasteuria*, and *Micrococcus* sp., effectively biomineralized Pb and/or Fe, Ni, Cu, Co, Zn, Cd, Fe through the MICP mechanism (Jiang et al., 2019; Kim et al., 2021; Li et al., 2013; Li et al., 2016a; Liu et al., 2021; Xue et al., 2022; Zeng et al., 2021).

*Sporosarcina pasterii*, obtained from various culture collections, is especially efficient precipitating lead and other toxic metals in the laboratory. In cultures containing 10 mM to 50 mM lead nitrate, 95–97% of the Pb was precipitated (Jiang et al., 2019). Similar results were observed when *S. pasterii* was grown in 0.01–1 mM lead acetate; Cu (60%), Zn (30%), Cd (43%), and Sr (100%) also were removed from solution (Kim et al., 2021). *S. pasterii* precipitated 100% Pb and Cd from landfill leachate, supplemented with lead nitrate (25 mg/L) and cadmium chloride (5.6 mg/L; Zeng et al., 2021). When a complex gas plant soil, contaminated with Pb (352 mg/kg), Zn (235 mg/kg), and Cd (6.8 mg/kg), was inoculated with *S. pasterii* in an aqueous medium, 14–68% Pb, 34–79% Zn, and 70–86% Cd were removed, resulting in a decrease in toxicity as evidenced by a bacterial luminescence assay (Liu et al., 2021). Metabolically inactivated *S. pasteurii* also removed >90% available Pb (0.05 mM to 5 mM $PbCl_2$) by Day 7 of incubation. At concentrations of 0.05 mM to 0.5 mM ($PbCl_2$), this was accompanied by an increase in pH (>8) and reduction of $Ca^{2+}$ (>95%). However, decrease of $Ca^{2+}$ lagged at the higher doses (1mM to 5mM $PbCl_2$) and an increase in pH was not observed (Mugwar and Harbottle, 2016). This suggests involvement of an abiotic mechanism and/or spore regeneration.

It should be noted that optimizing urea hydrolysis and nitrate reduction, which generate $HCO_3^-$, can help achieve higher levels of carbonate precipitation. Optimized *Ralstonia eutropha* H16 urease and nitroreduction activities in solution, substantially improved calcium carbonate biomineralization (Zhu et al., 2019b). Optimized activity of periplasmic and membrane-bound nitroreductases in *Proteus mirabilis* 10B enhanced Pb precipitation resulting in higher removal efficiencies under anoxic (91%) and oxic (95%) conditions following incubation with 350 ppm lead acetate and 350 ppm mercuric chloride (Eltarahony et al., 2020). Lead was precipitated as $PbCO_3$, $Pb_2O$, and $CaPbO_3$. Not only does precipitate formation modulate $Ca^{2+}$ levels, but it also serves as a resistance mechanism that protects the cell by sequestering $Pb^{2+}$ and other toxic metals.

**4.3.2 Consortia isolates—**Consortia isolated from targeted remediation sites may be more adapted to the complex environmental chemical, physical and biological conditions and therefore, more effective in biomineralizing Pb and other toxic metals. A consortium isolated from mine discharge sediment contaminated with Pb, As, Cd, Cu and Zn, comprised of 95% *Sporosarcina spp*. and 3% *Acidovorax spp*., precipitated Pb as observed by scanning electron microscopy (Proudfoot et al., 2022). A microbial consortium, isolated from coal mine drainage contaminated with Pb, Cd, Co, Ni, As, Fe, and Cr, was comprised of Proteobacteria and Bacteroidetes, which constituted 51% and 19% of the Bacteria, respectively, and Ascomycota and Cilophora, which accounted for 61% and 13% of the eukaryotes identified (Oyetibo et al., 2021). Seven taxonomic groups, were identified from the coal mine drainage as phylum Proteobacteria (class Gammaproteobacteria) *Acinetobacter pittii* group (Moraxellaceae), Enterobacteriaceae group (Enterobacteriaceae), unclassified FWNZ species (Enterobacteriaceae), and *Pseudomonas citronellolis* group (Pseudomonadaceae); and phylum Firmicutes (class Bacilli) *Sporosarcina koreensis* group (Planococcaceae), *Bacillus cereus* group (Bacillaceae) and *Exiguobacterium aurantiacum* group (Exiguobacteriaceae). The consortium was urease positive and formed metal-carbonate precipitates which negatively correlated with metal concentrations.

## 5. Environmental Factors for Lead Sequestration

Sequestration as phosphate-, sulfide- and carbonate precipitates are viable options for Pb and toxic metal sequestration and have the potential to decrease Pb bioavailability. While

PSB has been demonstrated for field applications with contaminated soils (Bradham et al., 2018; Park et al., 2011b), MICP and SRM have primarily been used under carefully controlled conditions (Kim et al., 2021; Sato et al., 2022). However, MICP cementing and soil stabilization properties, have been demonstrated in the field (Gomez et al., 2015; Phillips et al., 2016), suggesting the potential of this microbial process for use as an environmental remediation tool for Pb and other metals. New, more sustainable biomineralization technologies under investigation include incorporating sandstone waste (Sharma et al., 2022), carbide sludge (Yu and Zhang, 2023), or sulfate reducing bacteria containing granules (Chetty et al., 2022). Generating a biological based material that can withstand various matrices, such as freshwater, saltwater, and wastewater results in greater material versatility with a lower carbon footprint that can be employed in more remote locations (Chetty et al., 2022; Yu and Rong, 2022). Field application of these new technologies may inform future Pb sequestration field efforts.

While many microbial strains and consortia isolated for phosphate solubilization, sulfate reduction, or biogenesis of carbonate are Pb tolerant, their metabolic capacity to immobilize Pb is dependent on microbial strains and the environment. The oxygen status and pH are perhaps the most relevant (Fig. 6). For example, strict aerobes cannot survive in anoxic conditions and thus, cannot generate Pb sulfide. While facultative anaerobes have a wider range of oxygen tolerance, their ability to immobilize Pb under anoxic conditions may be limited because low oxygen is not metabolic favorable. The oxidation-reduction potential is dependent on physical, chemical, and biological factors. Because redox impacts compound solubility, it influences and is modulated in part by microbial community cycling of P, S, and C and other elements, all of which are inextricably intertwined (Peralta et al., 2014; Song et al., 2008). By changing the redox potential, Meng et al. (2019) identified a microbial driver of Cd sequestration in the family Anaerolineaceae, that influenced both microbial community composition and Cd solubility, thus having implications on bioaccessibility and a potential candidate to promote Cd sequestration in environmental bioremediation applications. In poorly buffered sediments, increasing oxygen content decreased pH and mobilized Pb and Cu; low pH (3–4.5) resulted in mobilization of more Pb and Cu in oxic compared to anoxic condition (Calmano et al., 1993). Redox cycling, i.e., cycles of anoxic-oxic condition, also influenced Pb, Zn, and Cd sulfide and carbonate formation and dissolution in sediment. In an effort to promote metal sulfide production during mine waste remediation, Karna et al. (2016) explored supplementing with organic carbon to decrease the oxygen concentration through respiration, and thus reduce the redox potential. This provided a more favorable environment for Pb-, Z-, and Cd-sulfide production.

Theoretically, these three precipitation mechanisms might occur simultaneously under anoxic conditions, yet usually one process is optimized for a specific application. Anaerobic conditions (dissolved oxygen <0.1 mg/L; oxygen-reduction potential, −65mV - −198mV) are required for sulfide production from sulfate; phosphatase activity and organic acid production (products dependent on pH) can occur under anoxic conditions however phosphatase activity is lower in the absence of oxygen (Horiuchi et al., 2002; Steenbergh et al., 2011). *Bacillus megaterium* formed calcium carbonate under anoxic (dissolved oxygen = 0.73–0.93 mg/L) conditions in the absence of Pb, so it is feasible that $PbCO_3$ could be produced by a Pb resistant strain (Jiang et al., 2016). Urease is active under anaerobic conditions, which further supports that $PbCO_3$ formation could occur anaerobically (Wozny et al., 1977). Most applications used to immobilize Pb with PSB and MICP are done aerobically where phosphate or carbonate are more efficiently produced. However, in a $CaCO_3$ rich environment, addition of organic carbon resulted in the formation of both galena (PbS) and cerussite ($PbCO_3$) as observed by synchrotron-based x-ray diffraction spectroscopy; cerussite only was detected in the absence of the organic carbon supplement (Karna et al., 2016).

Immobilization of Pb also is dependent on pH. Pyrophosphate forms at pH 5.3–10.0 (Liang and Lu, 1952), Pb sulfide at pH 7.5–8.5 (Hoa et al., 2007), and Pb carbonate at pH 7.5–9.5 (Xue et al., 2022). In solution with rock phosphate as a phosphate source, *Enterobacter* sp. produced organic acids that decreased the pH to 3.8, thus solubilizing rock phosphate; acid and alkaline phosphatases also were active (Park et al., 2011a; Park et al., 2011b). At low pH (2.2–3.2), tertiary Pb species were not formed and most remained solubilized because phosphate formation was inefficient. However, due to the buffering effect of soil constituents, the pH decreased to 5.3–5.5 which was conducive for pyrophosphate formation in soil. Conversely, ureolytic bacteria such as *Enterobacter cloacae* KJ-46 and KJ-47, produced carbonate and ammonia from urea, raising the pH >9.0 and removing 54–68% Pb from solution as $PbCO_3$ (Kang et al., 2015; Kang and So, 2016). Overall, weakly acidic-neutral pH is preferable for Pb phosphate formation whereas Pb carbonate generation occurs in alkaline conditions. When SRM is used to treat acid mine waste, increase the pH with lime or other alkaline material is required prior to inoculation of SRM. Adapting processes that leverage the natural ability of the microbial constituents to maintain pH should reduce the engineering needed to sequester Pb.

## 6. Future Perspectives

Use of biomineralization and biosorption leverages microbial metabolism and processes to produce recalcitrant transformation products and sequester Pb and other heavy metals. As one looks to the future of microbe-based remediation of environmental Pb, which will likely evolve considerably, several research needs are identified. First, further understanding the growth conditions of microbes and the associated molecular mechanisms responsible for Pb immobilization will inform future engineering applications that maximize Pb and other toxic metal removal from environmental systems. Modern molecular techniques play a key role in such research. DNA microarrays have been used to study the expression of genes under different environmental conditions, such as temperature, salinity and pH, and metabolomics has been used to study Pb induced changes, such as the oxygen stress response (Gao et al., 2017). Metagenomic analysis, or “bioprospecting”, will allow for molecular insight into changes in the microbial community structure, enrichment of related functional abundances e.g., metal metabolism, and activation of functional genes along with Pb immobilization (Li et al., 2022; Wani et al., 2022). A list of such functional genes and enzymes involved in phosphate, sulfide, and carbonate metal precipitation is summarized in Table 5. With these tools in hand, not only can important information on the niche differentiation of microbes be obtained, but they also can be used to predict their behavior in real world applications, thereby allowing for improved performance of Pb immobilization. The novel opportunities that have been created by the genomics revolution will generate enormous opportunities for microbiologists to obtain detailed insights into the ecology and biotechnology of these important microorganisms. Molecular manipulation has potential for desired trait selection which may facilitate a significant process change in Pb and toxic metal remediation.

Second, a multi-disciplinary approach is called for breakthroughs in the science and technology for microbial assisted remediation of Pb and toxic metals in the environment. While a biologist may lead microbial research, it is difficult to break the present obstacles using only microbial approaches. A coordinated effort in integrating biological and molecular theories with advanced analytical techniques and new findings from related disciplines such as material sciences, chemical engineering, and environmental sciences will allow for innovations and breakthroughs to advance the science and technology. One line of research towards this direction exhibits novel use of solid matrices embedded with microorganisms to immobilize metals (Wang et al., 2019a; Yang et al., 2019). For example, the vitality of microorganisms and enzymes can be maintained in alginate or nanomaterials such as hydroxyapatite impregnated polyurethane foam, which effectively bound Pb (Jang et al., 2008; Sone et al., 2009). More recently, metal-organic frameworks have been explored as remediation alternatives. Lead (667–833 mg/g) and Cd (370–714 mg/g) were removed from aqueous solution using two different magnetic metal-organic frameworks, $Fe_3O_4$@UiO-66–$NH_2$, and $Fe_3O_4$@ZIF-8 (Abdel-Magied et al., 2022); graphene oxide adsorbed 555 mg/g Pb (Bilal et al., 2021; Jun et al., 2019). Nanostructured materials with immobilized enzymes, such as laccases, showed potential for remediation of wastewater and other environmental contaminants.

Third, most studies reviewed in this paper were conducted in laboratory settings. Scaling up for environmental applications requires further investigation because the ambient environment and competition from the indigenous microbial community may have a large role in the functionality of the bacterial consortia or isolates. While carefully controlled pilot runs at the bench level are informative, in situ immobilization will be wrought with uncontrolled variables that need to be managed. Thus, optimization of the microbe functionalities for phosphate solubilization, sulfate reduction, or carbonate synthesis should be evaluated with a wide array of metrics relating to the environmental conditions in the field. Furthermore, biomineralization may form stable Pb precipitates while bio-sorbed Pb can be desorbed depending on the environmental condition. When selecting a process to reduce Pb bioavailability, the physical and chemical properties of the environmental matrix will inform the approach for Pb immobilization and microbial strain or consortium selection. Each aqueous, soil, or sediment matrix is unique, and the process will need to be optimized. Environmental conditions are influenced by physical, chemical, and biological factors which have an effect on the fate and transport of Pb. In a study by Karna et al. (2016), Pb-, Zn-, and Cd-sulfide were the preferred transformation products because they were more stable than their carbonate counterparts which were more likely to form in a $CaCO_3$ rich environment (PbS, 0.01244 g/100 mL vs $PbCO_3$, 0.00011 g/100 mL water at 20 °C). Therefore, the redox potential was manipulated by added organic carbon to drive respiration and remove oxygen from the system to enhance sulfide production. Additionally, because the transformation products are pH sensitive, low pH can lead to solubilization of the phosphate, sulfide, and carbonate compounds as discussed in Section 5, e.g., Pb phosphate, < pH 5.3 (Liang and Lu, 1952), Pb sulfide pH < 7.5 (Hoa et al., 2007), and Pb carbonate at pH < 7.5

(Xue et al., 2022). Biosorption of Pb also is pH dependent (Choińska-Pulit et al., 2018). Clearly, optimizing system biological, physical, and chemical parameters is paramount to a successful outcome, however some of the variables cannot be manipulated.

Furthermore, introduction of a single isolate optimized for a specific function may be met with intense competition from the highly adapted microbial residents, even if it were isolated from a similar contaminated site. For example, urease activity alone for MICP doesn't necessarily equate with efficacy due to complex environmental variables. This is evidenced by one study where, in the absence of toxic metals, a ureolytic *Sporosarcina pasteurii* isolate from a culture collection did not persist in soil as long as the community isolated from that soil (Graddy et al., 2021). On many occasions, consortia comprised of several functional species, especially those isolated from contaminated sources, may be a more viable alternative than a single isolate because in theory the consortium has adapted to the harsh environment and successful remediation would not be dependent on one specific isolate's survival. However, maintaining the percentages of the individual members of the community may be difficult, even though they are fully characterized. Some of the contaminated sites are over 100 years old and the existing microbial community is well adapted to the environment (Beattie et al., 2018). Consideration should be given to enriching for those resident microorganisms that have the functionalities needed to better sequester environmental Pb and other heavy metals.

## 7. Conclusions

Biological formation of Pb phosphate, sulfide, and carbonate precipitates has been demonstrated to provide effective approaches to immobilize Pb in environmental media such as soil, water and sediment. Each of the approaches addressed in this review has proven useful to immobilize Pb, thus making it less bioavailable, which in turn, reduces exposure to humans, animals, plants and the environment. Because microbial treatment is less harmful to the environment and more cost effective than chemical treatment or physical removal of contaminated soil or water off-site, results on microbial consortia and single isolate applications for phosphate solubilization, sulfate reduction, and MICP exhibit a novel strategy for remediation of Pb and other heavy metal contaminated sites. While many of the approaches are successful under carefully controlled laboratory settings, field application requires optimization for a host of variables to define a wide array of bio-engineering parameters, including microbial competitiveness, soil physical and chemical parameters, concentration, and co-contaminants. This is especially so for environmental factors such as redox potential and pH that have great impacts on microbial induced solubilization or immobilization, microbial activity, product (crystal) stability, and source availability. Large scale applications of microbe-based remediation will benefit from further multidisciplinary research to establish the effectiveness of bioremediation technology, particularly long-term monitoring of microbial survival, establishment, and transport for successful in-situ bioremediation of contaminated soil, water, and sediment.

## Acknowledgements

We would like to thank Dr. Richard Devereux for valuable reviews of earlier versions of this manuscript. We also thank Ashley Frith for her assistance in compiling the references. The views expressed in this paper are those of the

authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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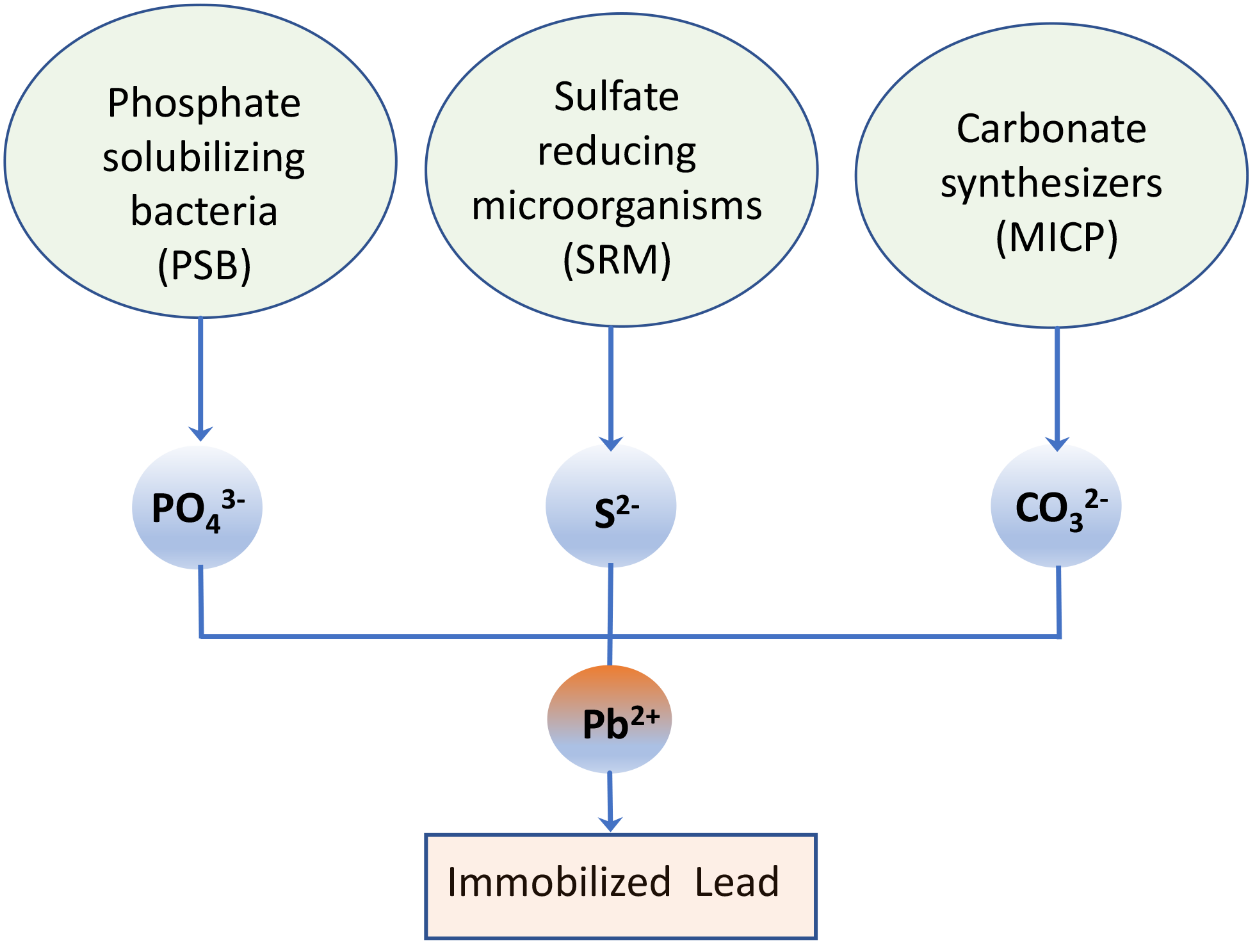

**Fig. 1.**

Schematic representation of immobilization of environmental lead mediated by microorganisms through phosphate solubilization, sulfate reduction, and microbially induced carbonate precipitation (MICP).

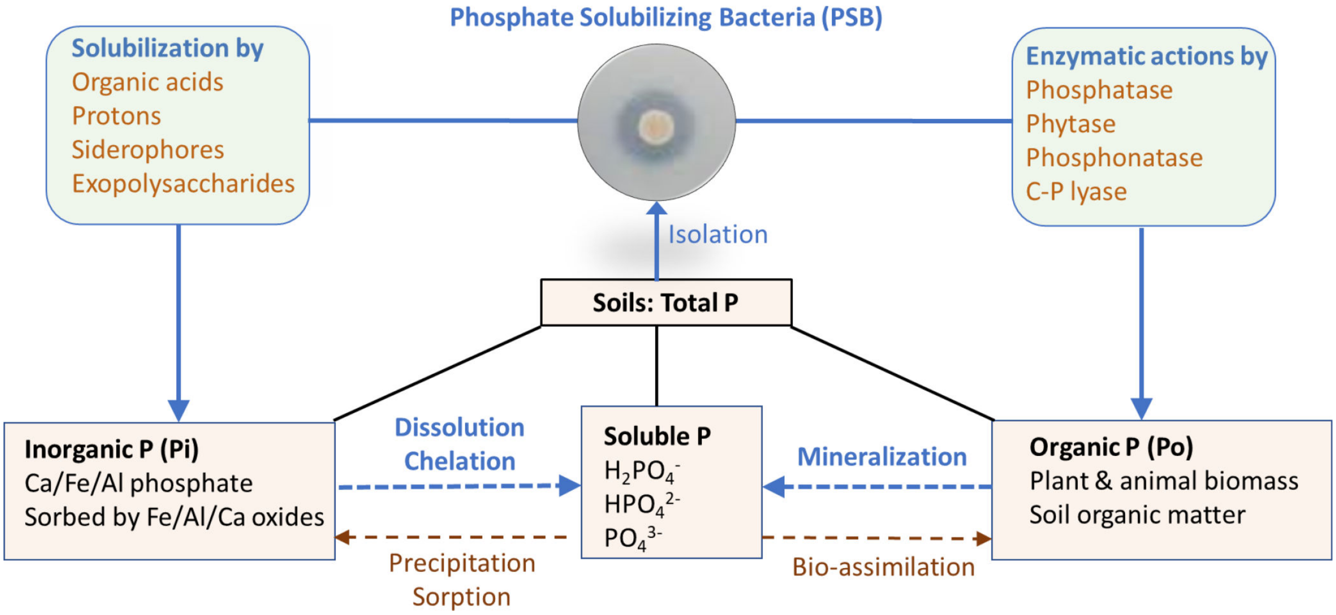

**Fig. 2.**

Soil phosphorus transformation and mechanisms of inorganic and organic phosphate solubilization by microorganisms.

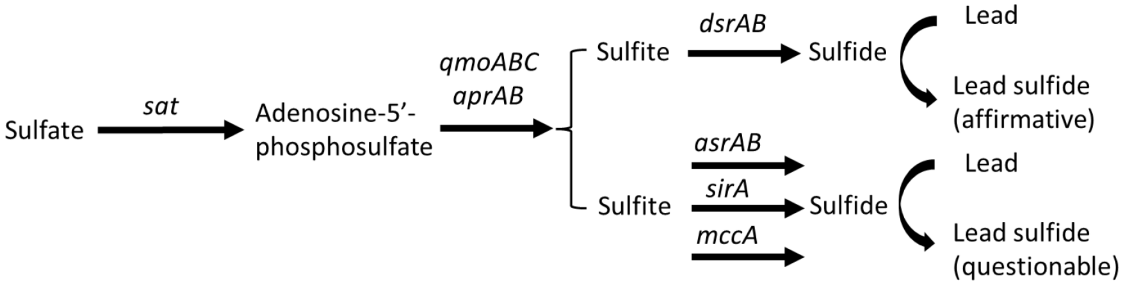

**Fig. 3.**

Dissimilatory sulfate and sulfite reduction for lead sulfide production. The functional genes involved include *sat*, sulfate adenylyl transferase (Fritz et al., 2000); *qmoABC*, quinone-interacting membrane-bound oxidoreductase (Ramos et al., 2012; Zane et al., 2010); *aprAB*, adenylyl sulfate reductase (Friedrich et al., 2002); *dsrAB*, dissimilatory sulfite reductase (sulfate reducing bacteria; Wagner et al., 1998); *asrA*, anaerobic sulfite reduction (*Salmonella typhimurium*; Huang and Barrett, 1991); *sirA*, cytochrome C sulfite reductase (*Shewanella oneidensis*; Shirodkar et al., 2011); *mccA,* cytochrome C sulfite reductase (*Wolinella succinogenes*; Eller et al., 2019; Kern et al., 2010).

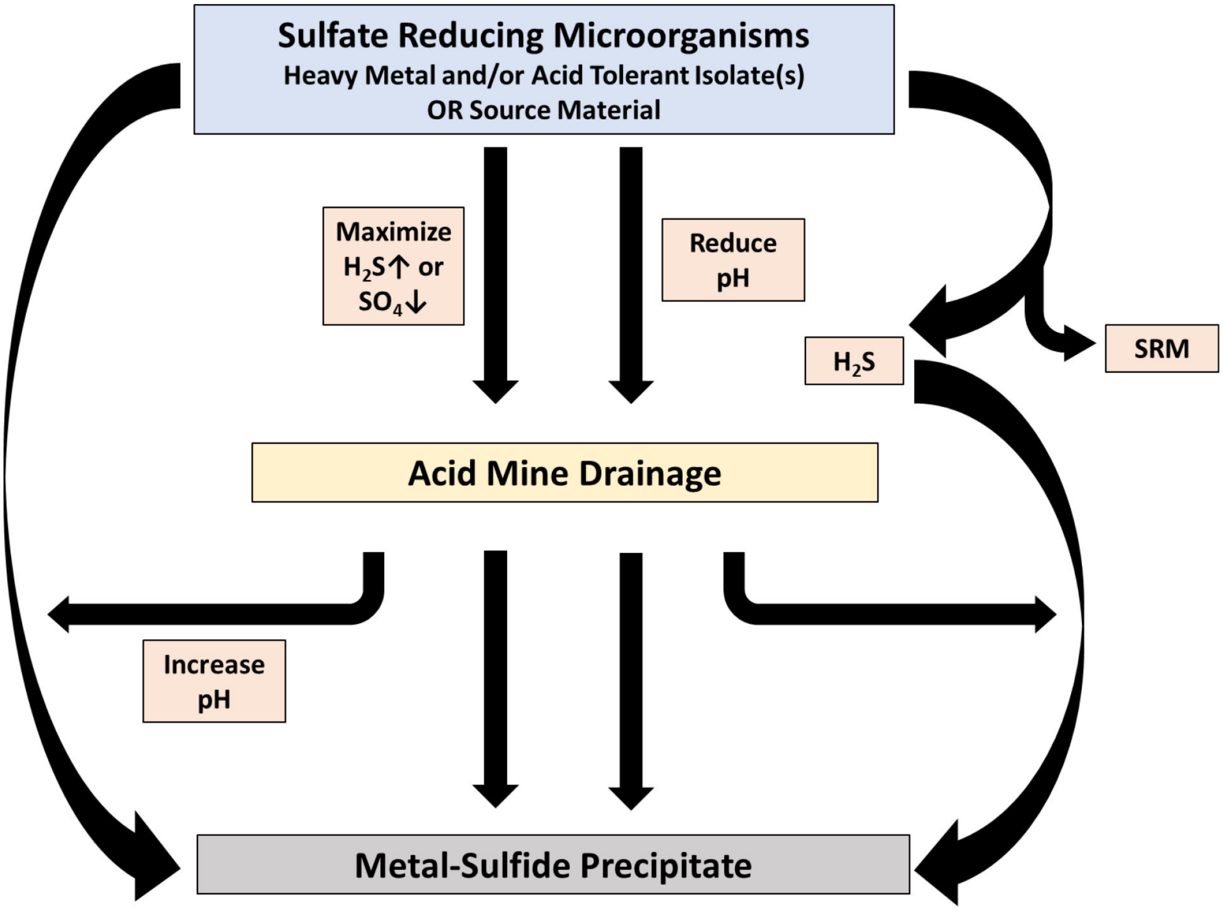

**Fig. 4.**

Applications that enrich for sulfate reducing microorganisms (SRM) or sulfide production in acid mine drainage and immobilization through metal sulfide precipitation.

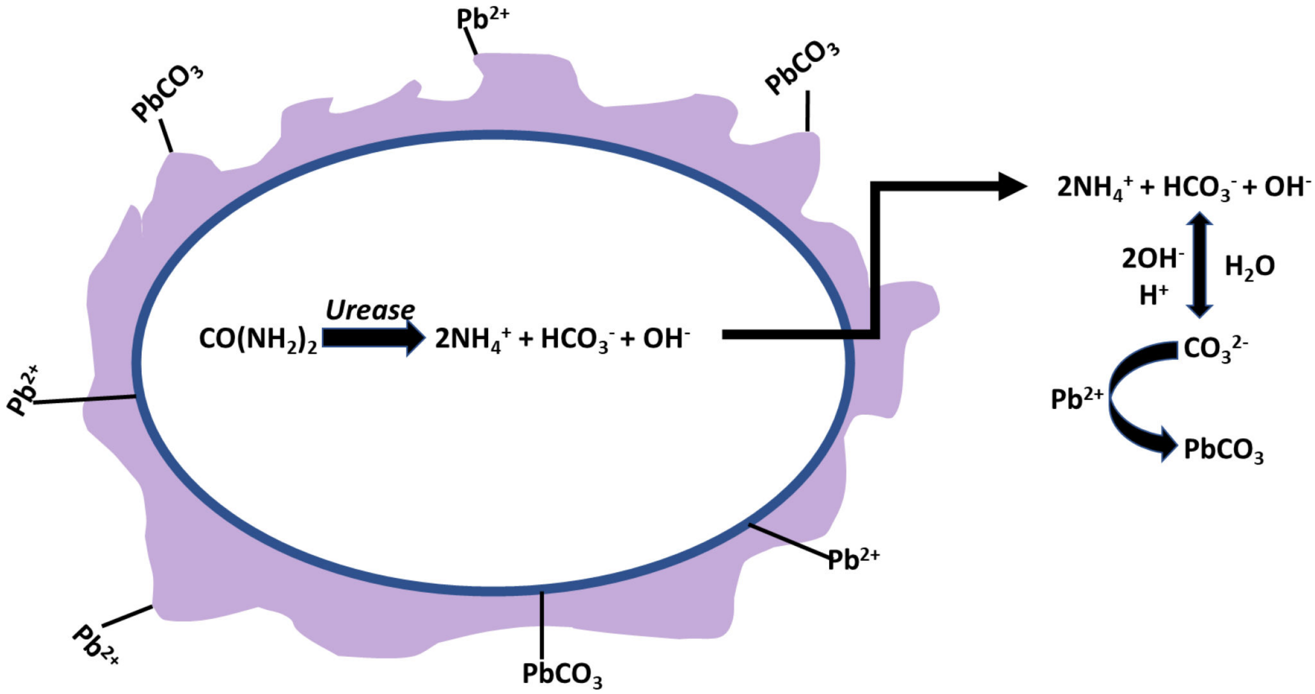

**Fig. 5.**

Urease mediated biogenesis of carbonate and for lead immobilization through biomineralization and biosorption with a bacterium cell. Purple shade represents exopolysaccharide produced by the bacterium and blue line is the cell wall.

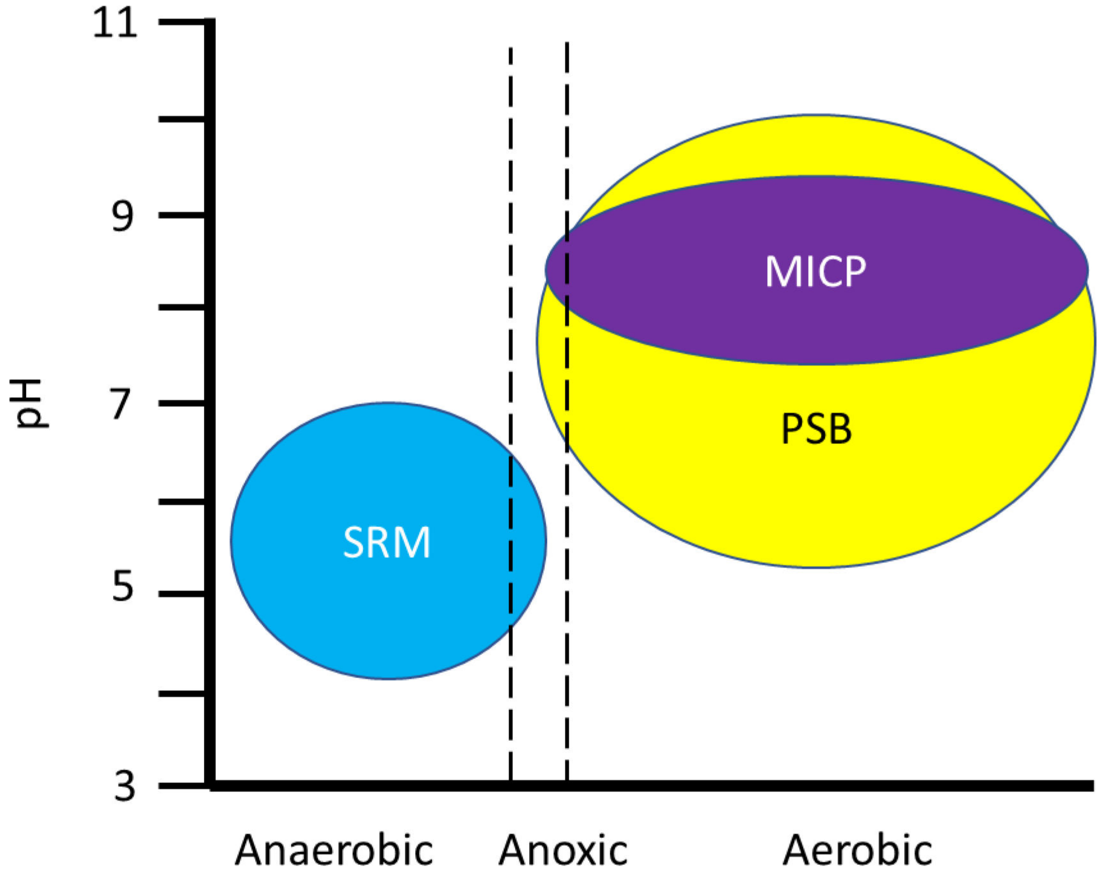

**Fig. 6.**

Oxygen and pH range for microbial mediated formation of lead sulfide, phosphate, and carbonate formation. PSB - phosphate solubilization bacteria, SRM -sulfate reducing microorganisms, and MICP - microbially induced calcium carbonate precipitation.

## Table 1.

Summary results of selected studies on immobilization of lead by phosphate solubilizing bacteria (ND, not determined)

| Source | Microorganism | Lead Resistance | P Solubilization capacity (mg/L) | P Source | P solubilization effects | Pb immobilization mechanism | References |
|---|---|---|---|---|---|---|---|
| China:<br>Pb-Zn concentrator tailing-waste contaminated soil:<br>Pb, 43.8 mg/kg<br>Cd, 0.33 mg/kg<br>Cu, 11.9 mg/kg | *Serratia marcescens* OPDB3–6-1 | 1.9 mM | 167.6 (15 d incubation) | Organic P in growth media | Production of alkaline matter<br>pH increase (7.0->8.5) | Precipitation Biosorption<br>Pyromorphite ($Pb_5(PO_4)_3Cl$) | Zhu et al., 2019 |
| China:<br>Soybean rhizosphere | *Enterobacter* sp. | 4.8 mM<br>Pb [1000mg/L] added to culture after 3 days incubation | ~160 (3 d incubation) | Organic P in growth media and biochar | Production of alkaline matter<br>pH increase (7.2-->8.7) | Precipitation: Pyromorphite ($Pb_5(PO_4)_3Cl$), cerussite<br>Biosorption (biochar) | Chen et al., 2019 |
| China:<br>Heavy metal waste collection & distribution center soil<br>Pb, 137.3 mg/kg (site L1)<br>Pb, 323.7 mg/kg (site F2) | *Leclercia adecarboxylata* L1–5<br>*Pseudomonas putida* F2–1 | 8 mM $Pb(NO_3)_2$<br>8 mM $Pb(NO_3)_2$<br>Minimum Inhibitory Concentration (MIC) > 1mM | 218 and ~18, respectively (7 d incubation) | $Ca_3(PO4)_2$ liquid medium | Production of organic acid (Acetate, Malonate) and acid phosphatase<br>pH decrease (6.7->2.8) | Precipitation:Hydroxylpyromorphite$Pb_{10}(PO_4)_6(OH)_2$ and Pyromorphite $Pb_5(PO_4)_3Cl$Biosorption (cell wall)<br>• Hydroxyl<br>• Amide<br>• Carboxyl<br>• Phosphate | Teng et al., 2019 |
| The Netherlands:<br>Canal water*<br>U.S.A.:<br>Human skin‡ | *Citrobacter freundii* ATCC 8090†<br>*Staphylococcus aureus* K4‡ | 1 mM $Pb(NO_3)_2$<br>7 mM $Pb(NO_3)_2$ | ND | ND | ND | ND | Levinson and Mahler, 1998<br>Levinson et al., 1996<br>Werkman and Gillen, 1932 |
| China:<br>Fertilizer plant soil | *Bacillus megaterium* | 100–500 mg/L $Pb^{2+}$ add to log phase cells | ND | Organic P in growth medium | ND | Precipitation/biosorption<br>Pb phosphate<br>$Pb_3(PO4)_2$<br>($Pb_5(PO_4)_3OH$ intermediate) | Zhang et al., 2019 |
| India:<br>battery manufacturing industry waste | *Achromobacter xylosoxidans* SJ11 | Maximum Tolerance Concentration (MTC): 4 mM $Pb(NO_3)_2$ | ND | Organic P in growth medium | 160% increase in phosphatase activity;<br>CheZ & CheA up-regulated | Precipitation: Pyromorphite $Pb_5(PO_4)_3Cl$<br>Extracellular accumulation | Sharma et al., 2018 |

| Source | Microorganism | Lead Resistance | P Solubilization capacity (mg/L) | P Source | P solubilization effects | Pb immobilization mechanism | References |
|---|---|---|---|---|---|---|---|
| China:<br>Heavy metal contaminated site (Pb/Zn smelting)<br><br>Pb, 127 mg/kg<br>Zn, 379 mg/kg<br>Cu, 93.1 mg/kg<br>Ni, 54.6 mg/kg<br>Co, 26.2 mg/kg<br>Mn, 1095 mg/kg<br>Cr, 133 mg/kg<br>V, 206 mg/kg<br>Hg, 0.15 mg/kg<br>As, 16.2 mg/kg<br>Cd, 3.08 mg/kg | PSB Consortium: *Enterobacter* spp., 92.65% *Bacillus* spp., 4.90% *Lactococcus* spp., 1.65% | 500 mg/kg $Pb(NO_3)_2$<br>400 mg/kg $CdCl_2$<br><br>MTC < 500 mg Pb/L | 429.5 | $Ca_3(PO4)_2$ in growth medium | ND | Precipitation<br><br>Biosorption<br><br>Intracellular accumulation | Yuan et al., 2017a; Yuan et al., 2017b |
| Australia:<br>Pb and Zn smelter site soil<br><br>Pb, 619 mg/kg | *Enterobacter sp.* | MIC = 2.23 mM $Pb(NO_3)_2$ | 200.3 after 14 d | Rock phosphate added into growth medium | Production of organic acids<br><br>pH decrease (7->3.8)<br><br>Increase in acid and alkaline phosphatase activities | Precipitation<br><br>Pyromorphite $Pb_5(PO_4)_3Cl$<br><br>Biosorption | Park et al., 2011a; Park et al., 2011b |
| Spain:<br>decayed wood | *Penicillium chrysogenum* | MIC = 8 mM $Pb(NO_3)_2$ | ND | Organic P in growth medium | ND | Precipitation/bio-sorption<br><br>Biotic:<br>Pb phosphate<br>$Pb_3(PO4)_2$<br><br>Abiotic:<br>Pb oxide ($Pb_3O_4$, PbO)<br>Hydrocerussite<br>$Pb_3(CO_3)_2(OH)_2$<br>Cerussite<br>$PbCO_3$ | Povedano-Priego et al., 2017 |
| China:<br>Root of Zn/Cd hyperaccumulator *Sedum alfredii* Hance, collected from Pb/Zn mine tailings (*Pantoea ananatis*) | *Pantoea ananatis* HCR2<br>*Bacillus thuringiensis* GL-1 | 3–5 mM Pb | 99.6 and 139.1<br><br>After 6–8 d | phosphate rock added into growth medium | Organic acids<br><br>Strain HCR2: gluconate, citrate, succinate, α-ketoglutarate, pyruvate<br><br>Strain GL-1: gluconate, citrate, α- | Precipitation<br><br>Strain GL-1: $Pb_5(PO_4)_3Cl$, $Pb_3(CO_3)_2(OH)_2$, $PbAl_3PO_4SO_4(OH)_6$, $Pb_3(CO_3)_2(OH)_2$<br><br>Strain HCR2: $Pb_5(PO_4)_3Cl$, $Pb_3(CO_3)_2(OH)_2$ | Xu et al., 2019 |

| **Source** | **Microorganism** | **Lead Resistance** | **P Solubilization capacity (mg/L)** | **P Source** | **P solubilization effects** | **Pb immobilization mechanism** | **References** |
|---|---|---|---|---|---|---|---|
| | | | | | ketoglutarate, succinate<br>pH decrease (6.7->2.8) | | |
| China: phosphate mining wasteland. | *Citrobacter farmer* CFI-01 in Pikovskaya's liquid medium | ND, at a minimum 100 mg Pb/L | 351.5 mg/L after 5 d | Calcium phosphate | pH reduced from 7.0 to 4.9 | Precipitation<br>$(Pb_5(PO_4)_3OH)$ $(Pb_5(PO_4)_3Cl)$ | Li et al., 2022 |

## Table 2.

Enhanced lead immobilization by inoculation of phosphate solubilizing bacteria in lead contaminated soils

| Phosphate solubilizing bacteria | Contaminated soil | P amendment rate | Lead immobilization measurement | Enhanced lead immobilization over the control without PSB inoculation | References |
|---|---|---|---|---|---|
| *Pantoea* sp. | Spiked with $Pb(NO_3)_2$ at 2000 mg Pb/kg soil | Rock phosphate amendment at 0, 200, 800 mg/kg soil | $NH_4NO_3$ (1M) extractable Pb concentration after 14 day incubation | 8.25–13.7% | Park et al., 2011a |
| *Enterobacter* sp. | Spiked with $Pb(NO_3)_2$ at 2000 mg Pb/kg soil | Rock phosphate amendment at 0, 200, 800 mg/kg soil | $NH_4NO_3$ (1M) extractable Pb concentration after 14 day incubation | 4.7–26.4% | Park et al., 2011a |
| PSB Consortium:<br>*Enterobacter* spp., 92.65%<br>*Bacillus* spp., 4.90%<br>*Lactococcus* spp., 1.65% | 435 mg Pb/kg soil taken at a Pb/Zn smelting site | $Ca_3(PO_4)_2$ amendment at 10.6 mg/g soil | Acetic acid (0.11 M) extractable Pb concentration after 15 day incubation | 10.81% | Yuan et al., 2017 |
| *Citrobacter farmeri* CFI-01 | Spiked with $Pb(NO_3)_2$ at 60 and 100 mg Pb/kg soil | Soil taken from phosphate mining site with total P of 2 mg/kg. No external P was added | $NH_4NO_3$ (1M) extractable Pb concentration after 14 day incubation | 7.79% and 22.18%, | Li et al., 2022 |
| *Acinetobacter pittii* gp-1 | Spiked with $Pb(NO_3)_2$ at 500 mg Pb/kg soil | 1, 2, 3, 4, 5, and 10% $Ca_3(PO_4)_2$ | Acetic acid (0.11 M) extractable Pb concentration after 30 day incubation | 8–22% | Wan et al., 2020 |

**Table 3.**

Summary results of selected studies on removal of Pb and other heavy metals by sulfate reducing bacteria. All identified are from the phylum Proteobacteria.

| Source | Microorganisms | Lead Concentration | Reactor | Removal Efficacy | References |
|---|---|---|---|---|---|
| USA:<br>Culture collection | *Desulfovibrio desulfuricans* G20 | 30 mg $L^{-1}$ | Flat-plate flow reactor with hematite or quartz containing sulfate reducing biofilm, pH 7.2 | 100% Pb + hematite<br><100% Pb + quartz | (Beyenal and Lewandowski, 2004) |
| Vietnam:<br>Industrial wastewater from battery storage | Consortium:<br>*Desulfovibrio vulgaris*<br>*Desulfovibrio carbinolicus**<br>*Desulfobacterium autotrophicum*<br>*Desulfomicrobium salsugmis*<br>*Desulfocmicrobium escambiense**<br>*dominant | Loading rate 20, 30, 40 mg$^{l-}$day$^{-1}$ Pb<br>Stock 0, 100, 150, 200 mg $L^{-1}$ | Moving bed biofilm reactor, pH 7.5 optimum (pH 6.5–8.5) | 99–100% Pb | (Kieu et al., 2015)<br>(Nguyen et al., 2017) |
| China:<br>Sewage treatment plant landfill leachate | *Desulfobulbus propionicus*<br>*Desulfovibrio vulgaris* | Cu, 17 mg $L^{-1}$<br>Cd, 9.4 mg $L^{-1}$<br>Zn, 131 mg $L^{-1}$<br>Pb, 38 mg $L^{-1}$<br>(Sediment) | Reaction bottle<br>+/- micro zero valent iron (mFe$^0$)<br>pH 6.2–7.5 | 100% Cu<br>98.5% Cd<br>90.7% Zn<br>100% Pb | (Li et al., 2016b) |
| Thailand:<br>Anaerobic lagoon treating tapioca wastewater | Unidentified Sulfate Reducing Bacteria & Methane Producing Bacteria | 45–50 mg $L^{-1}$ Pb | Two-Stage Reactor<br>1) Sulfide production (Anaerobic sludge blanket reactor, pH 7.5–8.5)<br>2) Lead precipitation (Chamber containing $Pb(NO_3)_2$; no SRB present) | 85–95% Pb | (Hien Hoa et al., 2007)<br>7.5–8.5 optimum 85–95% Pb removal |
| The Netherlands:<br>Domestic wastewater anaerobic sludge | Consortium:<br>Not identified | Cu, 10 mg $ml^{-1}$<br>Zn, 10 mg $ml^{-1}$<br>Pb, 10 mg $ml^{-1}$<br>Cd, 10 mg $ml^{-1}$ | Inversed fluidized bed reactor, influent pH 7.0, effluent pH 6.6–7.5 | 98.4% Cu<br>96.5% Zn<br>96% Pb<br>97.9% Cd | (Villa-Gomez et al., 2011) |
| China:<br>Culture collection | Not Identified | Cu, 7.1 mg $kg^{-1}$<br>Zn, 339.6 mg $kg^{-1}$<br>Pb, 12.7 mg $kg^{-1}$<br>Cd, 80.2 mg$kg^{-1}$<br>(Sediment) | SRB immobilized on polyvinyl alcohol beads, pH7.2 | 91.2% Cu<br>95.6% Zn<br>100% Pb<br>76.3% Cd | (Li et al., 2017) |

## Table 4.

Summary results of selected studies on immobilization of lead by microbial induced carbonate precipitation.

| Source | Microorganism (Phylum) | Metal Tolerance | Precipitate | Immobilization Efficacy | Reference |
|---|---|---|---|---|---|
| | **Bacteria** | | | | |
| United Kingdom:<br>Culture collection | *Sporosarcina pasteurii* (Firmicutes) | $PbCl_2$, 1.0 mM<br>$ZnCl_2$, 0.5 mM<br>$CuCl_2$, 0.5 mM<br>$CdSO_4$, 0.06 mM | $PbCO_3$<br>$CaCO_3$<br>Biotic/Abiotic<br>~pH9.0 | | (Mugwar and Harbottle, 2016) |
| USA:<br>Culture collection | *Sporosarcina pasterii* (ATCC 6452) | $Pb(NO_3)_2$, 10–40 mM | $(PbCl)_2CO_3$<br>PbCl(OH)<br>$PbCO_3$<br>$Pb_3(CO_3)_2(OH)_2$<br>$CaCO_3$<br>$PbCl_2$ (Abiotic) | | (Jiang et al., 2019) |
| South Korea:<br>Culture collection | *Sporosarcina pasterii* (KCTC 3558) | $Pb(CH_3COO_2)$, 0.01–1 mM<br>$CuCl_2$, 0.01–1 mM<br>$ZnCl_2$, 0.01–1 mM<br>$Cd(C_2H_3O_2)_2$, 0.01–1 mM<br>$SrCl_2$, 1–30 mM | $PbCO_3$ (Cerussite)<br>$CaCO_3$ (Aragonite, Calcite, Vaterite)<br>pH >9.0<br>$ZnCO_3$ pH >8.5<br>$CdCO_3$ pH >9.0<br>$(Sr,Ca)CO_3$ pH>9.0 | >99% Pb<br>30% Zn<br>60% Cu<br>43.2–60% Cd<br>>99% Sr | (Kim et al., 2021) |
| Germany:<br>Culture collection | *Sporosarcina pasteurii* (DSM 33) | Pb, 342 mg $kg^{-1}$<br>Zn, 235 mg $kg^{-1}$<br>Cd, 6.8 mg $kg^{-1}$ | $PbCO_3$<br>$CaCO_3$<br>$ZnCO_3$<br>$CdCO_3$ | | (Liu et al., 2021) |
| USA:<br>Culture collection | *Sporosarcina pasterii* (ATCC 11859) | $Pb(NO_3)_2$, 25 mg/L<br>$CdCl_2$, 5.6 mg/L | $Pb_3(CO_3)_2(OH)_2$ (Hydrocerussite)<br>$PbCO_3$<br>$CdCO_3$<br>$CaCO_3$ (Calcite) | | (Zeng et al., 2021) |
| China:<br>Culture collection | *Sporosarcina pasterii* | $PbCl_2$, 10–50 mM | | ~100% Pb | (Xue et al., 2022) |
| China & USA:<br>Culture collection & garden soil | *Sporosarcina pasteurii* ATCC 11859<br>*Sporosarcina globispora UR53*<br>*Sporosarcina Koreensis UR47*<br>*Sporosarcina sp. R-31323 (UR31)*<br>*Terrabacter tumescens* AS.1.2690 (Actinobacteria) | $PbCl_2$, 2 mg $ml^{-1}$<br>$NiCl_2$, 2 mg $ml^{-1}$<br>$CuCl_2$, 2 mg $ml^{-1}$<br>$CoCl_2$, 2 mg $ml^{-1}$<br>$ZnCl_2$, 2 mg $ml^{-1}$<br>$CdCl_2$, 2 mg $ml^{-1}$ | $PbCO_3$<br>$NiCO_3$<br>$CuCO_3$<br>$CoCO_3$<br>$ZnCO_3$<br>$CdCO_3$<br>pH 8–9 | 100% Pb<br>88% Ni<br>91% Cu<br>91% Co<br>95% Zn<br>97% Cd | (Li et al., 2013) |

| Source | Microorganism (Phylum) | Metal Tolerance | Precipitate | Immobilization Efficacy | Reference |
|---|---|---|---|---|---|
| | **Bacteria** | | | | |
| | *Bacillus lentus UR41*<br>(Firmicutes) | | | | |
| South Korea:<br>Abandoned mine soil | (*Firmicutes*)<br>*Sporosarcina soli* B-22<br>*Viridibacillus arenosi* B-21<br>*Lysinibacillus sphaericus* KJ-64<br>*Sporsacina pasteurii* WJ-2<br><br>(Proteobacteria, Gamma-)<br>*Enterobacter cloacae* KJ-46 | $PbCl_2$, 3000 mg $L^{-1}$<br>$CoCl_2$, 300–3000 mg $L^{-1}$<br>$CoSO_4$, 300–3000 mg $L^{-1}$<br>$CuSO_4$, 300–3000 mg $L^{-1}$<br>$CuCl_2$, 300–1000 mg $L^{-1}$<br>$FeCl_3$, 3000–1000 mg $L^{-1}$<br>$FeSO_4$, 300–3000 mg $L^{-1}$<br>$CdCl_2$, 300–3000 mg $L^{-1}$<br>$BaCl_2$, 2000–3000 mg $L^{-1}$<br>$SrCl_2$, 3000 mg $L^{-1}$<br>$ZnSO_4$, 300–3000 mg $L^{-1}$ | $PbCO_3$<br>$CaCO_3$<br>$C_4CuO_4$<br>$Zn(OH)_2$ | | (Kang and So, 2016) |
| Mexico:<br>Mine tailings (mg/kg)<br>As, 1140–11,800<br>Pb, 10100–43,700<br>Zn, 780–10,000<br>Cd, 8–780<br>Cu, 72–1320<br>Fe, 6000–12,300 | *Sporosarcina luteola* (UB3 & UB5) | $As^{V}$, $Cr^{VI}$, $Fe^{3+}$, $Fe^{2+}$, $Co^{2+}$, $Rb^{+}$ $Sb^{3+}$; 25–50 mM<br>$Mn^{2+}$, $As^{III}$, $Cu^{2+}$, $Ni^{2+}$, $Ba^{2+}$; 5–10mM<br>$Pb^{2+}$, $Cd^{2+}$, $Zn^{2+}$, $Hg^{2+}$, $Te^{4+}$, $Ag^{+}$; <1mM | $PbCO_3$ (Cerussite)<br>$CaCO_3$ (Calcite, Vaterite)<br>$MnCO_3$ (rhodochrosite)<br>$Mg_5(CO_3)_4(OH)_2$ (hydromagnesite)<br>$CdCO_3$ (otavite)<br>$SrCO_3$ (strontianite)<br>$BaCO_3$ (witherite)<br>$Zn_5(CO_3)_2(OH)_6$ (hydrozincite)<br><br>pH 8.7–9.0 | | (Cuaxinque-Flores et al., 2020) |
| USA:<br>Mine discharge sediment<br>$\sim10^2$ mg/L As<br>$\sim10^1$ Cd<br>$\sim10^3$ Cu<br>$\sim10^3$ Zn | Consortium<br>*Sporosarcina* spp., 95%<br>*Acidovorax* spp*., 3%*<br>(Proteobacteria, -Beta) | $Pb^{2+}$, $\sim10^3$-$10^4$ mg $L^{-1}$ | Precipitation (not identified)<br><br>pH>8.0 | | (Proudfoot et al., 2022) |
| China:<br>Culture collection | *Terrabacter tumescens* A12<br>(Actinobacteria) | $PbCl_2$, 2 mg $ml^{-1}$<br>$NiCl_2$, 2 mg $ml^{-1}$<br>$CuCl_2$, 2 mg $ml^{-1}$<br>$CoCl_2$, 2 mg $ml^{-1}$<br>$ZnCl_2$, 2 mg $ml^{-1}$<br>$CdCl_2$, 2 mg $ml^{-1}$ | $PbCO_3$<br>$CaCO_3$<br>$NiCO_3$<br>$CuCO_3$<br>$CoCO_3$<br>$ZnCO_3$<br>$CdCO_3$<br><br>pH 9.0 | 99% Pb<br>90% Ni<br>90% Cu<br>91% Co<br>97% Zn<br>99% Cd<br>99% Ca | (Li et al., 2016a) |
| China:<br>Electronic waste | *Lysinibacillus* sp. (GY-3) | $PbCl_2$, 20–1000 ppm<br>$PbCl_2$, 20–200 ppm<br>$CuCl_2$, 20–100 | Precipitation (not identified) | 87.5–100% Pb<br>80.6–98.7% Cu | (Li et al., 2021) |

| Source | Microorganism (Phylum) | Metal Tolerance | Precipitate | Immobilization Efficacy | Reference |
|---|---|---|---|---|---|
| | **Bacteria** | | | | |
| China:<br>Mining area soil | *Kocuria flava* CR1<br>(Actinobacteria) | $Pb(NO_3)_2$, 50 mM<br>Pb, 100 mg $kg^{-1}$ | $PbCO_3$<br>CaCO3 (Calcite, Vatarite) | 83.4% Pb | (Achal et al., 2012) |
| South Korea:<br>Pb contaminated mine tailings soil<br>Pb, 1050 mg/kg Zn, 431 mg/kg<br>Cu, 93 mg/kg<br>As, 65 mg/kg | *Bacillus* sp. KK1 | $Pb(NO_3)_2$, 300 mg/L<br>$ZnCl_2$, 150 mg/L<br>$CuCl_2$, 650 mg/L<br>As, 150 mg/L<br>(Mine tailing soil) | $PbCO_3$<br>$PbSiO_3$ (Alamosite)<br>PbS | | (Govarthanan et al., 2013) |
| China:<br>Lake water | *Exiguobacterium* sp. (JBHLT-3; Firmicutes) | $PbCl_2$, 1mM | $PbCO_3$<br>$CaCO_3$ (Calcite, Vaterite<br>(saltwater) | 89% Pb | (Bai et al., 2021) |
| South Korea:<br>Abandoned metal mine soil | *Enterobacter cloacae KJ-47 & KJ-46* | 100 Mm $PbCl_2$ (MIC) | $PbCO_3$ | 60% Pb | (Kang et al., 2015) |
| Japan:<br>Soil near beachrock | *Pararhodobacter* sp.<br>(Proteobacteria, -Alpha) | $PbCl_2$, 1036 mg $L^{-1}$ | $PbCO_3$<br>$CaCO_3$ (Calcite & Vaterite)<br>Biotic/Abiotic<br>pH 8.8 | 100% PB | (Mwandira et al., 2017) |
| Egypt:<br>Soil | *Micrococcus sp.* NCTC-1716 or WD-9<br>(Actinobacteria) | $Pb(NO_3)_2$, 5 mM<br>$CdSO_4$, 2mM<br>$ZnCl_2$, 3 mM<br>$FeSO_4$, 3 mM | $PbCO_3$<br>$CaCO_3$<br>$ZnCO_3$<br>$CdCO_3$<br>$FeCO_3$<br>pH 9 | 61% Cd<br>97% Pb<br>75% Zn<br>88% Fe | (Gomaa, 2019) |
| China:<br>Heavy metal contaminated soil from industrial area | *Staphylococcus epidermis* HJ2<br>(Firmicutes) | $PbCl_2$, 50 mg $l^{-1}$<br>$K_2Cr_2O_7$, 50 mg $l^{-1}$ | $PbCO_3$<br>$CaCO_3$<br>pH >9.0 | 86% Pb<br>76.8% Cr | (He et al., 2019) |
| Egypt:<br>Culture collection | *Proteus mirabilis (10B)* | Pb, 350 ppm<br>Hg, 350 ppm | $PbCO_3$<br>$Pb_2O$<br>$CaPbO_3$<br>$Hg_2O$<br>(Aerobic/Anaerobic)<br>pH 8.4 | 95.2% Pb<br>92% Hg<br>98.4% Ca | (Eltarahony et al., 2020) |
| | Bacteria & Eukaryota | | | | |
| Nigeria:<br>Coal mine drainage | Proteobacteria (51%)<br>Bacteroidetes (19%) | Pb, 326 mg $L^{-1}$<br>Cd, 95.0<br>As, 307.6 | Heavy metal carbonate precipitates<br>pH >8.2 | 94.8% Pb<br>96.3% Cd<br>88.9% As | (Oyetibo et al., 2021) |

| Source | Microorganism (Phylum) | Metal Tolerance | Precipitate | Immobilization Efficacy | Reference |
|---|---|---|---|---|---|
| | **Bacteria** | | | | |
| | Ascomycota (61%)<br>Ciliophora (13%) | Ni, 28.8<br>Co, 27.3<br>acid mine drainage | | 90.6% Ni<br>27.3% Co | |
| Egypt:<br>Culture collection | *Metschnikowia pulcherrima (29A)*<br>*Raoultella planticola* (VIP; Proteobacteria, Gamma-)<br>*Alcaligenes faecalis* (46N)<br>*Bacillus aryabhattai* (39A)<br>*Ochrobactrum sp.* (CNE2)<br>*Streptomyces cyaneofuscatus* (EM3) | VIP & 29A: $Pb(CH_3COO)_2$ & $HgCl_2$, 700 ppm<br>39A, 46N, EM3, CNE2: inhibited by $Pb(CH_3COO)_2$ & $HgCl_2$, 175 ppm | $CaPbO_3$ $PbCO_3$ (Cerussite)<br>$CaHgO_2$<br>HgO | 100% Pb<br>100% Hg<br>>95% Ca | (Eltarahony et al., 2021) |
| | Eukaryota | | | | |
| Australia:<br>Karstic cave | *Aspergillus sp. (UF3)*<br>*Fusarium oxysporum* (UF8) | $PbNO_3$, 10–100 mM<br>$SrCl_2$, 10–100 mM | $PbCO_3$<br>$Pb_2OCO_3$<br>$CaCO_3$ (Aragonite, Vaterite, Calcite)<br>$SrCO_3$ | | (Dhami et al., 2017) |
| China:<br>Contaminated cement sludge<br>Pb, 366 mg/kg<br>Cr, 91.4 mg/kg | *Penicillium chrysogenum* (CS1) | $Pb(CH_3COO)_2$, 400 mg $l^{-1}$<br>$K_2Cr_2O_7$, 100 mg $l^{-1}$ | $Pb_3(CO_3)_2(OH)_2$ (Hydrocerussite)<br>$CaCO_3$ (Calcite & Vaterite)<br>Calcium chromium oxide carbonate<br>pH 9.22 & 8.47 | 98% Pb<br>39–65% Cr | (Qian et al., 2017) |

## Table 5.

Representative genes and enzymes involved in phosphate, sulfide, and carbonate metal precipitation

| Gene | Enzyme | Microorganism | Reference |
|---|---|---|---|
| | | **Phosphate** | |
| *phoD* | alkaline phosphatase | *Acinetobacter pittii* | Wan et al., 2020 |
| *phyK* | histidine acid phosphatase | *Xanthomonas campestris* | Huang et al., 2009 |
| | | *Pseudomonas syringae* | Cangussu et al., 2018 |
| | | *Pectobacterium carotovorum* (formerly *Erwinia carotovora*) | Cangussu et al., 2018 |
| *phyA* | beta-propeller phytase | *Acinetobacter pittii* | Wan et al., 2020 |
| | | *Xanthomonas oryzae* pv. *oryzae* | Chatterjee et al., 2007 |
| *pqq* | pyrroloquinoline-quinone synthase | *Acinetobacter pittii* | Wan et al., 2020 |
| *pkk* | polyphosphate kinase | *Acinetobacter pittii* | Wan et al., 2020 |
| | | **Sulfide** | |
| *sat* | sulfate adenylyltransferase | *Thiorhodovibrio* | Vavourakis et al., 2019 |
| | | *Thioalkalivibrio* sp. | Vavourakis et al., 2019 |
| | | *Thohalocapsa* sp. | Vavourakis et al., 2019 |
| *aprAB* | adenylyl sulfate reductase alpha & beta subunits | *Desulfovibrio* spp. | Ramos et al., 2012 |
| *qmoABC* | quinone-interacting membrane-bound oxidoreductase | *Desulfovibrio* spp. | Ramos et al., 2012 |
| *dsrAB* | dissimilatory sulfite reductase alpha & beta subunits | *Desulfovibrio vulgaris* | Oliveira et al., 2008 |
| | | *Thiorhodovibrio* | Vavourakis et al., 2019 |
| | | *Thioalkalivibrio* sp. | Vavourakis et al., 2019 |
| | | *Thohalocapsa* sp. | Vavourakis et al., 2019 |
| | | *Ca. Rokubacteria* | Anantharaman et al., 2018 |
| | | *Ca. Hydrothermarchaeota* | Anantharaman et al., 2018 |
| *sirA* | dissimilatory sulfite reductase | *Shewanella oneidensis* | Shirodkar et al., 2011 |
| *mccA* | dissimilatory sulfite reductase | *Wolinella succinogenes* | Kern et al., 2011 |
| *asrAB* | siroheme-dependent anaerobic sulfite reductase | *Ca. Woesearchaeota* | Vavourakis et al., 2019 |
| | | *Salmonella spp.* | Vavourakis et al., 2019 |
| | | *Edwardsiella spp.* | Vavourakis et al., 2019 |
| | | **Carbonate** | |
| *ureC* | Urease subunit alpha | *Sporosarcina luteola* | Cuaxinque-Flores et al., 2020 |

| Gene | Enzyme | Microorganism | Reference |
|---|---|---|---|
| | | **Phosphate** | |
| | | *Sporosarcina pastuerii* | Kang & So, 2016 |
| | | *Viridibacillus arenosi* | Kang & So, 2016 |
| | | *Enterobacter cloacae* | Kang & So, 2016 |
| | | *Lysinibacillus sphaericus* | Kang & So, 2016 |