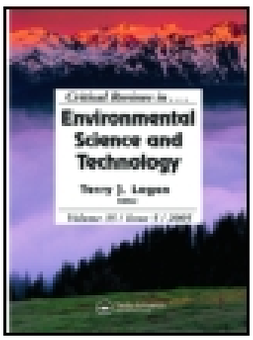

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Advances in characterizing microbial community change and resistance upon exposure to lead contamination: Implications for ecological risk assessment

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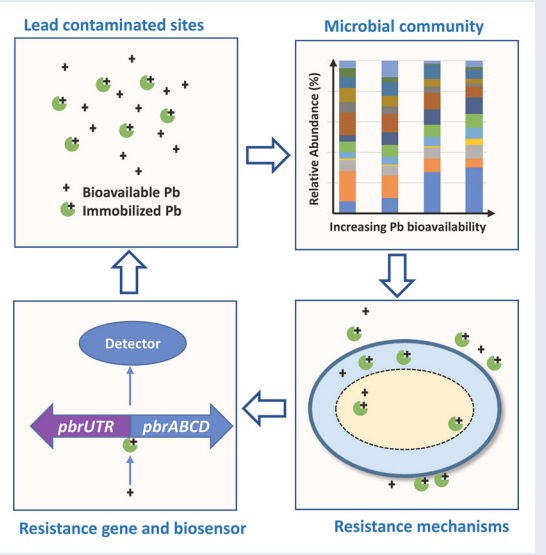
ABSTRACT



Recent advancement in molecular techniques has spurred numerous studies on responses of microorganisms to lead exposure, leveraging detailed phylogenetic analyses and functional gene identification to discern the effects of lead toxicity on microbial communities. A comprehensive review of recent research is provided on (1) lead resistance mechanisms of microorganisms; (2) microbial community changes in contaminated aquatic sediments and terrestrial soils; and (3) lead resistance genes applied to lead biosensor development. Ample evidence in the literature, including both *in vitro* and *in situ* studies, indicates that exposure to lead inhibits microbial activities (such as respiration and metabolism), reduces biomass and alters microbial community structure. Even at sites where microbial communities do not vary compositionally with contaminant levels, functional differences between microbial communities are evident. The main mechanisms of lead resistance involve extracellular and intracellular biosorption, precipitation, complexation, and/or efflux pumps. The suites of genes associated with lead resistance mechanisms can serve, when considered with phylogenetic information, as indicators of lead contamination. This holds potential for development of next generation lead biosensors. To promote applications of advanced knowledge, molecular techniques, and lead biosensor technology, perspectives on using microbial indicators for site ecological assessment are presented.

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KEYWORDS Ecological risk assessment; lead biosensors; lead contamination; lead resistance; microbial community

1. Introduction

Environmental and occupational exposure to lead (Pb) continues to cause health effects globally, primarily through exposure to contaminated air, water, food, soils and household dust (Tong, von Schirnding, & Prapamontol, 2000; WHO, 2011). Lead reserves are estimated at 7.1 x 10^7 tons in ores such as galena (PbS), anglesite ($PbSO_4$) and cerussite ($PbCO_3$), and over the past three centuries, largely due to industrialization, environmental Pb levels have significantly increased (U.S. DHHS/ATSDR, 2007). Lead is mined worldwide and purified through smelting, which releases Pb into the environment in the form of hazardous fumes, fallout, and dust (World Health Organization & International Agency for Research on Cancer (WHO/IARC), 2006; U.S. DHHS, 2007). Cores from the Muztagata glacier (at 2,100 m altitude) in Pamirs have revealed temporal Pb concentrations from Central Asia in part due to industrial operations such as smelting (Li et al., 2006). Total Pb concentrations on contaminated sites can exceed 10,000 mg/kg, while the average value in natural soils ranges from <10 to 50 mg/kg. Von Braun et al. (2002) have reported environmental Pb levels in the Rudnaya Pristan-Dalnegork (Russian Federation) region as high as 95,000 mg/kg, primarily due to spillage of product during transportation (railroad ties were contaminated with up to 293,000 mg/kg). At a copper smelting operation in Karabash (Russian Federation), Pb contamination has been detected in soil (2,000 mg/kg), air (30 μg/m^3), and snowmelt (316 μg/L) as well as in children's hair and local vegetables (Revich, 2010; Udachin et al., 2003; Williamson et al., 2004). Lead contamination detected along a transect initiating at the Port Pirie (Australia) Pb smelting site revealed levels decreasing from 2,220 mg/kg adjacent to the site (2 km) to 140 mg/kg 33.5 km away (Cartwright, Merry, & Tiller, 1976). Significant levels of Pb have been detected in local children and grazing sheep (Koh & Judson, 1986; Maynard et al., 2003; Wilson, Esterman, Lewis, Roder, & Calder, 1986).

Lead can be found as a co-contaminant with other heavy metals such as arsenic (As), cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni) and zinc (Zn). In Ust-Kamenogorsk (Kazakhstan), Pb, Cd, Cu and Zn in soil reached 1,348, 27, 856 and 2,406 mg/kg, respectively (Woszczyk, Spychalski, & Boluspaeva, 2018). Breast milk from lactating mothers living in Karaganda and Balkhash (Kazakhstan) contained approximately 0.3 mg/L Pb and other heavy metals, exceeding admissible levels by 39-fold (Kulkybaev, Dyusembin, & Konkabaeva, 2002). The Port Pirie Pb smelter effluent containing Pb, Zn and Cd at 750, 4300 and 64 μg/L, respectively, was released into marine waters and eventually diluted (Ferguson, 1983).

Aside from mining and smelting activities, sequestered Pb is released into the environment from dredging activities and has been associated with oceanic micro- and macro-plastics (Nayar, Goh, & Chou, 2004; Yang et al., 2019). Lead is a component of many commercial products such as automobile batteries, solder, x-ray machine shielding, and corrosion resistant paints. In the United States, Pb was used as a paint pigment until 1978, and as Pb solder for food cans and an "anti-knock" agent in gasoline until 1995 (U.S. DHHS/ATSDR, 2007). Industrial waste can be contaminated with Pb and other heavy metals, which in turn pollute the surrounding environment (Jiang et al., 2019; Kuppusamy et al., 2016; Li et al., 2017; Shi, Bischoff, Turco, & Konopka, 2002). Banning Pb as a fuel additive resulted in decreasing air concentrations but, because Pb does not degrade in the environment, Pb based paint and contaminated soil remain public health concerns.

Lead causes neurological, cardiovascular, renal, hematological, gastrointestinal, musculoskeletal, endocrinological, immunological, reproductive and developmental effects and is listed as a probable human carcinogen (WHO, 2011; WHO/IARC, 2006; U.S. DHHS, 2007). Many countries regulate environmental Pb (Tong et al., 2000). In the United States, the Environmental Protection Agency (U.S. EPA) stipulates that the National Ambient Air Quality Standard for Pb is 0.15 $\mu g/m^3$ total suspended particles (Federal Register, 2008; 2016). The US EPA's standard for Pb in bare soil is 400 mg/kg for play areas and 1200 mg/kg for non-play areas (U.S. Environmental Protection Agency (U.S. EPA), 2001). Lead is frequently found as a co-contaminant at Superfund sites however there is no Pb reference dose (RfD). Cleanup of Pb contamination includes removal of material, groundwater treatment, engineering controls and other approaches focused on reducing the risk of Pb exposure, especially to children (U.S. EPA, 2019), consistent with the U.S. Federal Lead Action Plan (U.S. Executive Office of the President, 2018).

Bioaccessibility, bioavailability, bioaccumulation, and bioconcentration are all considered in a Pb risk assessment (U.S. EPA, 2007a, 2007b). Lead is not biologically essential and can be toxic to microorganisms. However, some microorganisms can develop a variety of resistance mechanisms that allow their survival in high Pb concentrations, and this leads to an increase in Pb resistance genes and changes in microbial community activity and composition (Braud, Hoegy, Jezequel, Lebeau, & Schalk, 2009; Naik & Dubey, 2011; O'Brien, Hodgson, & Buckling, 2014). Because microorganisms play a critical role in carbon (C) and nitrogen (N) cycling and other fundamental geochemical processes, groups of European scientists have advocated for ecological risk assessments that consider microbial effects (Dahlin, Witter, Martensson, Turner, & Baath, 1997; de Vries et al., 2007;

Giller, Witter, & McGrath, 2009). However, in the United States, microbial screening levels for ecological risk assessment were not recommended, partly due to considerable spatial and temporal variation of the effects and associated experimental uncertainties (U.S. EPA, 2003). Twenty-five years ago, studies to ascertain the impact of Pb and other heavy metals on microorganisms focused on measuring respiration, biomass, litter decomposition and enzyme activity to infer critical toxicity concentrations of Pb and other heavy metals (de Vries et al., 2007; Green, Stott, & Diack, 2006). Microbial community diversity was studied using microscopy, plate counting and phospholipid fatty acid (PLFA) analysis (Dahlin et al., 1997; Fakruddin & Mannan, 2013). While PLFA measurement is still used today to identify metal induced alterations in microbial community structure (Lenart & Wolny-Koładka, 2013; Zhang, Xu, et al., 2018; Zhang, Wan, et al., 2018), it does not provide detailed quantitative information about microbial community structure. Recent advancements in molecular techniques (e.g., 16S rRNA gene sequencing, functional gene microarray, metagenomic sequencing) coupled with statistical analyses (e.g., principle component analysis, canonical correspondence analysis, and network analysis) have allowed for detailed phylogenetic analyses, functional gene identification, and discernment of the effects of Pb and other heavy metals on microbial communities (e.g., Chen et al., 2018; Jacquiod et al., 2018). As these molecular approaches become more commonplace, higher resolution in microbial community composition is possible and microbial Pb resistance genes can be incorporated into Pb biosensors for detecting bioavailable Pb in a contaminated site (Hobman, Julian, & Brown, 2012; Zhang, Qin, Deng, & Wells, 2017).

Recent advances in molecular technology and analysis of microbial community and heavy metal resistance genes open the door for reconsidering the use of microorganisms in ecological risk assessment. Consequently, the objectives of this paper are 1) to further elucidate various Pb resistance mechanisms of microorganisms; 2) to evaluate the impact of Pb and other heavy metals on microbial community structure and function in contaminated terrestrial soils and aquatic sediments; and 3) to examine the use of Pb specific resistance genes for Pb biosensor development to detect Pb and assess Pb bioavailability. Perspectives on future work using microbial indicators for site ecological risk assessment are also presented.

2. Lead resistance mechanisms of microorganisms

Microorganisms have developed extracellular and intracellular strategies to persist in Pb contaminated environments, some of which are Pb specific and others act more general (reviewed by Jaroslawiecka & Piotrowska-

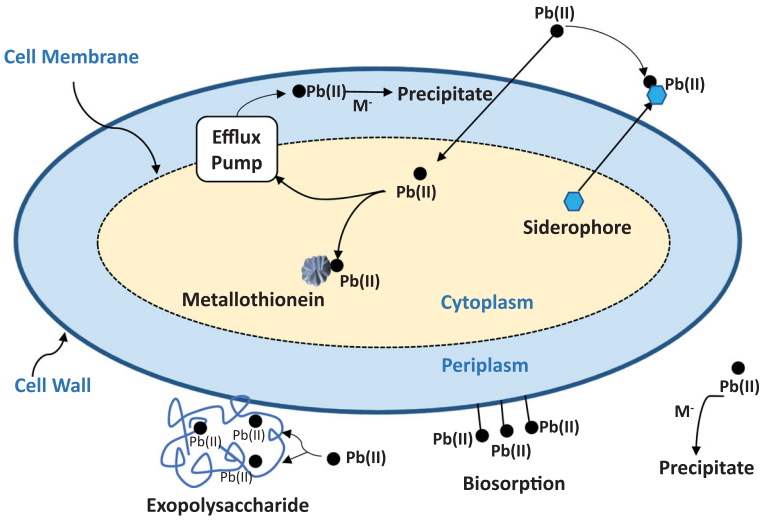

Figure 1. Schematic representation of lead resistance mechanisms operational in extracellular and intracellular spaces of a bacteria cell. "M^-" represents anions such as phosphate that precipitate lead ions, Pb(II).

Seget, 2014; Naik & Dubey, 2013; Pan et al., 2017). Lead can be sequestered extracellularly in exopolysaccharide matrices (De, Ramaiah, & Vardanyan, 2008; Macaskie & Dean, 1987; Naik, Pandey, & Dubey, 2012a; Nelson, Lo, Lion, Shuler, & Ghiorse, 1995; Roane, 1999) or scavenged by excreted siderophores (Naik & Dubey, 2011; O'Brien et al., 2014), ultimately resulting in Pb precipitates, such as Pb phosphates through phosphatase action. Surface biosorption (lipopolysaccharide, Gram negative bacteria; peptidoglycan, Gram positive bacteria) also excludes Pb from the cell (Chang, Law, & Chang, 1997; Karimpour et al., 2018). Lead can enter the cell and either bind with metallothionein (Murthy, Bali, & Sarangi, 2011; Naik, Shamim, & Dubey, 2012c), or, through an efflux mechanism, be transported by P-type ATPase to the periplasm where phosphatase release of pyrophosphate results in Pb precipitation (Borremans, Hobman, Provoost, Brown, & van Der Lelie, 2001; Hynninen, Touze, Pitkanen, Mengin-Lecreulx, & Virta, 2009; Murthy et al., 2011; Naik et al., 2012c; Rensing, Sun, Mitra, & Rosen, 1998). These Pb resistance mechanisms are schematically shown in Figure 1. Several extracellular and intracellular strategies are described in detail below.

2.1. Extracellular immobilization

When exposed to Pb contamination, microorganisms can invoke extracellular immobilization strategies to limit the entry of Pb ion [Pb(II)] into the cell envelop to maintain metal homeostasis. Selected microbial species with extracellular biosorption functions reported in the literature are listed in

Table 1. Bacterial species with extracellular immobilization functions involving exopolysaccharide (EPS), siderophores and cell surface biosorption.

Isolation source	Pb(II) media	**Phylum**[a]	Genus/Species	References
Exopolysaccharide (EPS) production				
Coast	$Pb(CH_3COO)_2$ 100ppm	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	De et al., 2008
Lead battery manufacturing plant	$Pb(NO_3)_2$ 1.6 mM	*Gammaproteobacteria*	*Enterobacter cloacae*	Naik et al., 2012a
–	$Pb(C_2H_3O_2)_2$ 1.3–13 mg/L	*Gammaproteobacteria*	*Marinobacter* sp.	Bhaskar & Bhosle, 2006
–	–	*Betaproteobacteria*	*Burkholderia cepacia* (formerly *Pseudomonas cepacia*)	Nelson et al., 1995
–	$Pb(NO_3)_2$ 1mM	*Gammaproteobacteria*	*Citrobacter* sp.	Macaskie & Dean, 1987
Metal mine	$Pb(NO_3)_2$ 2.5 mM	*Gammaproteobacteria*	*Pseudomonas marginalis*	Roane, 1999
Industrial waste water	$Pb(NO_3)_2$	*Firmicutes*	*Bacillus anthracis*	El-Shanshoury et al., 2012
Siderophore production				
Car battery waste	$Pb(NO_3)_2$ 0.5mM	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	Naik & Dubey, 2011
–	$Pb(NO_3)_2$	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	O'Brien et al., 2014
Surface biosorption				
Hospital sewage	$PbCl_2$	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	Chang et al., 1997
Soil	–	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	Karimpour et al., 2018

[a]Proteobacteria are listed by class if available.

Table 1. In general, extracellular immobilization is accomplished mainly through biosorption and precipitation. While extracellular precipitation of Pb resembles intracellular precipitation, which is described in the next section, biosorptive binding of Pb involves a series of polymers or compounds produced by microorganisms including exopolysaccharides, siderophores, and various functional groups on the cell wall (Figure 1). Note that the biosorption of Pb(II) to exopolysaccharides, siderophores, or cell wall components is not specific to Pb.

Exopolysaccharides (EPS) are high molecular weight polyanionic polymers secreted by microorganisms into their environment. These extracellular polysaccharides protect the cell from Pb and other heavy metals by preventing their entry into the cell and providing an advantageous environmental niche for the EPS-coated cell and its sensitive neighbors that become enveloped with the EPS (Bitton & Freihofer, 1997). Transmission electron microscopy revealed that the Pb accumulation mechanism in *Pseudomonas marginalis* (Roane, 1999) and *Enterobacter cloacae* strain P2B (Naik et al., 2012a) was through binding to carboxyl, hydroxyl, and amide functional groups and glucuronic acid in EPS polymer chains. Glucuronic acid EPS was found to be produced constitutively in *Pseudomonas marginalis* (Roane, 1999), or induced in the presence of metals including Pb, in *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *Bacillus subtilis* subsp. *subtilis*, *Enterobacter cloacae* strain P2B and other *Enterobacter* spp. (Bhaskar & Bhosle, 2006; Chowdhury, Mishra, Adarsh, Mukherjee, & Thakur, 2008; El-Shanshoury, Elsilk, Ateya, & Ebeid, 2012; Naik et al., 2012a). A *Bacillus anthracis* strain isolated from industrial waste water was found to excrete EPS and precipitate Pb sulfide (PbS) extracellularly (El-Shanshoury et al., 2012). Purified EPS from *Marinobacter* sp. unexposed to Pb, bound Pb and Cu *in vitro* (Bhaskar & Bhosle, 2006). Immobilized biofilms, which contain polysaccharides and other biopolymers (reviewed by Marvasi, Visscher, & Casillas Martinez, 2010), accumulated Pb, a phenomenon enhanced by the addition of iron in *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) (Macaskie & Dean, 1987; Nelson et al., 1995). Inactivated *Pseudomonas aeruginosa* cells have been shown to bio-sorb Pb, possibly due to interactions with EPS (Chang et al., 1997; Karimpour et al., 2018).

Siderophores ("iron carrier", Greek) are small, high-affinity iron-chelating compounds secreted by microorganisms such as bacteria and fungi. Microorganisms excrete siderophores to chelate iron and transport it back across the cell membrane (Neilands, 1995). However, these same relatively low molecular weight compounds can also bind heavy metals, such as Pb, and reduce toxicity (O'Brien et al., 2014). In *Pseudomonas aeruginosa* PAO1, Pb bound to the siderophore pyoverdine but was not transported into the cell (Braud et al., 2009). Some heavy metals, such as Cd,

Co, gallium (Ga), Hg, Mn and Zn, inhibited iron uptake, however Pb did not (Braud et al., 2009). Furthermore, it did not inhibit pyoverdine production (Braud et al., 2009). A Pb resistant strain of *Pseudomonas aeruginosa* (strain 4EA), isolated from soil contaminated with Pb battery waste, increased pyochelin and pyoverdine production coupled with reduced cell size in the presence of Pb (Naik & Dubey, 2011). Excreted siderophores also scavenged Pb, resulting in the production of Pb precipitates through phosphatase action and formation of Pb phosphates (Naik & Dubey, 2011; O'Brien et al., 2014).

Organic functional groups such as hydroxyl, carboxyl, and nitrogen-, sulfur-, and phosphorus-containing groups on the cell wall can sorb Pb(II) onto the cell surface. This may occur either in living or in inactivated cells. Chang et al. (1997) noted that both inactivated cells and resting cells of *Pseudomonas aeruginosa*, isolated from sewage, could adsorb Pb (II) with high capacities. This is further confirmed by Karimpour et al. (2018) wth *Pseudomonas aeruginosa* isolated from contaminated soil. Scanning electron microscopy and energy dispersive X-ray spectroscopy revealed biosorption of Pb onto the cell surface of *Pseudomonas aeruginosa*, isolated from soil contaminated with Pb battery waste (Naik & Dubey, 2011). Because of their absorbent properties, these Pb resistance bacterial strains or isolated EPS potentially may serve as biosorbents for Pb remediation.

2.2. Intracellular accumulation

When Pb(II) enters the cell due to exposure to high Pb concentrations, intracellular resistance strategies are triggered. Initially, an efflux pump or Pb transporting ATPase, transports Pb from the cytoplasm through the cell membrane to the periplasm where Pb can form insoluble precipitates by oxidation or reduction reactions, thereby sequestering Pb and protecting the cell from its toxic effects. Additionally, metallothioneins also can bind Pb in the cytoplasm (Figure 1). Selected microbial species with intracellular accumulation through precipitation and binding with metallothioneins reported in the literature are listed in Table 2.

Precipitates identified in Pb-exposed microbes include Pb oxide (PbO; El-Shanshoury et al., 2012), Pb sulfide (PbS; De et al., 2008; El-Shanshoury et al., 2012; Essa, Al Abboud, & Khatib, 2018; Gong, Zhang, Bai, & Yang, 2007; Li, Peng, Jia, Lu, & Fan, 2016), Pb sulfite ($PbSO_3$; Sharma, Shamim, Dubey, & Meena, 2017), Pb sulfate ($PbSO_4$; Li et al., 2016), Pb carbonate ($PbCO_3$; Essa et al., 2018; Kang et al., 2015), Pb phosphate ($Pb_3(PO_4)_2$; Borremans et al., 2001; Hynninen et al., 2009), and an unusual Pb phosphate salt, $Pb_9(PO_4)_6$, precipitated by *Vibrio harveyi* (Mire, Tourjee, O'Brien, Ramanujachary, & Hecht, 2004). Lead precipitates typically are

Table 2. Bacterial species with intracellular Pb accumulation: precipitation or metallothionein binding.

Isolation source	Pb (II) media	**Phylum**[a]	Species	Precipitate/ Metallothionein gene	Reference
Precipitation					
Coast	$Pb(CH_3COO)_2$ 100ppm	*Actinobacteria* *Firmicutes*	*Brevibacterium iodinum* *Bacillus pumilus*	PbS	De et al., 2008
Sewage treatment plant	$Pb(NO_3)_2$ 300 mg/L	*Firmicutes*	*Desulfotomaculum sp.*	PbS	Gong et al., 2007
–	$Pb(NO3)_2$ 2.5 mM	*Gammaproteobacteria*	*Vibrio harveyi*	$Pb(PO_4)_6$	Mire et al., 2004
Sewage treatment wetland	$Pb(NO_3)_2$ 0.1mM	*Gammaproteobacteria* *Firmicutes* *Actinobacteria*	*Pseudomonas aeruginosa* *Bacillus amyloliquefaciens* *Bacillus subtilis* subsp. *subtilis* *Microbacterium luteolum*	Lead nanoparticles (mineral not identified)	Chowdhury et al., 2008, 2011
Leather workshop	$Pb(NO_3)_2$	*Gammaproteobacteria*	*Enterobacter sp.*	PbO	El-Shanshoury et al., 2012
Industrial waste water	$Pb(NO_3)_2$	*Firmicutes*	*Bacillus anthracis*	PbS	El-Shanshoury et al., 2012
–	$Pb(NO_3)_2$ 6 mM	*Gammaproteobacteria*	*Escherichia coli*	Lead particles	Essa et al., 2018
–	$Pb(NO_3)_2$ 4 mM	*Firmicutes*	*Staphylococcus aureus*	Lead particles	Essa et al., 2018
Sludge	$Pb(NO_3)_2$	*Gammaproteobacteria*	*Escherichia coli*	PbS & $PbCO_3$	Essa et al., 2018
Metal mine	$PbCl_2$ 100 mM	*Gammaproteobacteria*	*Enterobacter cloacae*	$PbCO_3$	Kang et al., 2015
Oil field injection water	$Pb(NO_3)_2$ 150 mg/L	*Proteobacteria, alpha*	*Rhodobacter sphaeroides*	PbS & $PbSO_4$	Li et al., 2016
Metal mine soil	$Pb(NO_3)_2$ 0.1 mM	*Firmicutes*	*Bacillus megaterium*	Mineral unidentified	Roane, 1999
Battery waste	$Pb(NO_3)_2$ 3.0 mM	*Gammaproteobacteria*	*Providencia vermicola*	$PbSO_3$	Sharma et al., 2017
Metallothionein (intracellular accumulation)					
Soil, car battery waste	$Pb(NO_3)_2$ 0.2-0.4 mM	*Gammaproteobacteria* *Gammaproteobacteria*	*Salmonella enterica* (formerly *Salmonella choleraesuis*) *Proteus penneri*	*smtAB*	Naik et al., 2012c
Estuary	$Pb(NO_3)_2$ 0.6 mM	*Gammaproteobacteria*	*Pseudomonas* sp.	*bmtA*	Naik et al., 2012b
Industrial effluent	$Pb(NO_3)_2$ 0-500 mg/L	*Firmicutes*	*Bacillus cereus*		Murthy et al., 2011

[a]Proteobacteria are listed by class if available.

formed in the periplasm, a space between the inner and outer membrane in bacterial cells. Intracellular Pb oxide (PbO) precipitation was reported to occur in the periplasm of an *Enterobacter sp*. (El-Shanshoury et al., 2012). Sharma et al. (2017) described periplasmic Pb sulfite accumulation in *Providencia vermicola* strain SJ2A, isolated from a battery manufacturing plant. Lead sulfide (PbS) has been associated with Pb exposed *Brevibacterium iodinium* GP13, *Bacillus pumilus* S3, *Escherichia coli* Z3, and *Rhodobacter sphaeroides* (De et al., 2008; Essa et al., 2018; Li et al., 2016) and accumulated in the periplasm of *Desulfotomaculum* sp. (Gong et al., 2007) and *Bacillus megaterium*, isolated from a silver mining region (Roane, 1999). Accumulation of periplasmic Pb phosphate by *Cupriavidus metallidurans* strain CH34 (formerly *Alcaligenes eutrophus, Wautersia metallidurans,* and *Ralstonia metallidurans*) has also been described (Borremans et al., 2001; Hynninen et al., 2009).

Lead is exported to the periplasm by an efflux mechanism with a P_{IB} family P-type ATPase from where it combines with pyrophosphate. P-type ATPase efflux has been associated with Pb resistance in *Escherichia coli* (*znt*A; Beard, Hashim, Membrillo-Hernández, Hughes, & Poole, 1997; Rensing, Mitra, & Rosen, 1997; Rensing et al., 1998), *Staphylococcus aureus* (*cad*A), and *Pseudomonas putida* (*cad*A2) (Hynninen, Tonismann, & Virta, 2010). *Pseudomonas aeruginosa* strains C1 and C2, *Bacillus amyloliquefaciens* strains F1, *Bacillus subtilis* subsp. *subtilis* strain F3, and *Microbacterium luteolum* strain GZ, isolated from a wetland inundated by sewage contaminated with toxic metals, harbored Pb containing nanoparticles intracellularly (Chowdhury et al., 2008; Chowdhury, Thakur, and Chaudhuri, 2011). These strains have been used to develop a bioremediation package for removal of Pb from Pb contaminated wastewater.

Intracellular accumulation of Pb precipitates has been associated with cell morphological changes such as shortening and thickening cells of *Pseudomonas aeruginosa* (Chowdhury et al., 2008), spheroidal shaped cells of *Desulfotomaculum* sp. (Gong et al., 2007), and interconnected filaments formed from *Providencia vermicola* strain SJ2A rod-shaped cells (Sharma et al., 2017). *Enterobacter* sp. (El-Shanshoury et al., 2012), *Pseudmononas aeruginosa*, *Bacillus subtilis subsp. subtilis* and *Bacillus amyloliquerfaciens* (Chowdhury et al., 2008; 2011) increased EPS secretion in association with Pb precipitation, thus providing a two-layer defense against Pb toxicity.

Metallothioneins are known to bind divalent metals and contribute to cellular metal homeostasis (Robinson et al., 1990). These cysteine-rich proteins are involved in Zn and Cd resistance (Naz, Young, Ahmed, & Gadd, 2005; Turner, Morby, Whitton, Gupta, & Robinson, 1993) and the literature has suggested that Pb also may be sequestered intracellularly by a metallothionein (Roane, 1999). Roane (1999) observed cytoplasmic and

periplasmic Pb accumulation in *Bacillus megaterium* by transmission electron microscopy and postulated both efflux and metallothionein involvement in Pb resistance. Cadmium localization was found to be similar in *Desulfivibrio desulfuricans* DSM 1926 and *Desulfococcus multivorans* DSM 2059. Cytoplasmic accumulation was linked to the *Synechococcus* PCC 7942 metallothionein *smt*AB gene homolog involved in Zn resistance, providing more supporting evidence of metallothionein involvement (Robinson et al., 1990; Naz et al., 2005). Cadmium, Zn and Cu treatment increased *smt*A and *smt*B transcript abundance and deletion mutations in the *smt*A gene, which is known to bind Zn and Cd, resulted in decreased Zn resistance (Robinson et al., 1990; Turner et al., 1993). Increased metallothionein production was correlated to Pb exposure in *Bacillus cereus* (Murthy et al., 2011). *Salmonella choleraesuis* strain 4 A and *Proteus penneri* strain GM10 were shown to harbor the *smt*A gene and bioaccumulate Pb, suggesting probable metallothionein involvement in Pb sequestration in these species (Naik et al., 2012). The gene *bmtA*, encoding a related bacterial metallothionein, was detected in *Pseudomonas aeruginosa* strain WI-1, accompanied by induction of a probable *bmtA* gene product (metallothionein protein) and intracellular sequestration of Pb (Naik, Pandey, & Dubey, 2012b). The *bmtA* gene has been described in *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Anabaena* PCC 7120 and binds Zn (Blindauer et al., 2002).

Note that binding of Pb to either siderophores or metallothioneins can result in a unique chemical signature. Instruments such as HPLC, HPLC coupled with ICP Mass Spectrometry or differential pulse polarography, and SDS-PAGE have been used to characterize microbial metallothionein or siderophores (Drewniak, Skłodowska, Manecki, & Bajda, 2017; Murthy et al., 2011; Naik et al., 2012b; Olafson, 1986; Takatera, Osaki, Yamaguchi, & Watanabe, 1994). Literature on chemical analysis of Pb-metallothionein complexes has not been found. Fluorescence of Pb-siderophore complexes produces a signal different from the unbound siderophore (Braud et al., 2009). Showing an increase in siderophores and/or metallothioneins by chemical analyses could infer presence of environmental Pb or other contaminants (Drewniak et al., 2017). Looking for increases in genes related to specific resistance mechanisms would be more informative and probably much easier than developing methods for Pb-siderophores or Pb-metallothioneins.

3. Impact of lead and other heavy metal contamination on microbial community structure and function

Understanding the effect of Pb and other heavy metal contamination on microbial communities, including activity and community composition, is important because microorganisms may serve as a direct or indirect

Table 3. Lead spiked soil impacts microbial metabolism and community composition.

Soil Source	Lead treatment	Microbial Effects[a]	Reference
Barossa Valley region, South Australia	5000 (4605; 92.1% recovery) mg/kg soil incubated with $Pb(NO_3)_2$ for 49 days	(−) Microbial respiration (CO_2 evolution) & biomass carbon (+) Gram negative bacteria & Gram positive bacteria (−) Fungi & Actinomycetes	Xu et al., 2018
Highly sodic yet low acidity (pH > 5.5) soils in the Barossa Valley region, South Australia	50 and 5000 mg/kg incubated with $Pb(NO_3)_2$ for 7 and 49 days	(−) Microbial activity (basal respiration, microbial biomass carbon, and microbial functional groups) (−) Microbial community compositions based on the microbial phospholipid fatty acids (PLFA) analysis: Greater negative influence on the fungal population than bacteria	Xu et al., 2019
Resembling Mississippi silty clay soil & Delta topsoil (Yazoo silty clay), Mississippi, USA	500, 1000, 2000 mg/kg incubated with $Pb(NO_3)_2$ at 18–24 °C for 18 months	Selected for Pb resistant nitrite reducers (*nirK*) Shift in microbial community (−) Community diversity	Sobolev & Begonia, 2008
Uncontaminated soil, Beijing, China	100 & 500 mg Pb/kg incubated with $Pb(NO_3)_2$ for 4 weeks	Dominant phyla (all soils): *Bacteroidetes*, *Proteobacteria*, *Firmicutes*, *Actinobacteria* On genus level[b]: *Firmicutes:* (+) *Bacillus* *Bacteroidetes:* (+) *Adhaeribacter, Pontibacter, Flavisolibacter* *Alphaproteobacteria:* (+) *Kaistobacter*	Liu et al., 2018
Hangzhou, China	2200, 400, 600, 800, 1000 mg/kg incubated with $Pb(NO_3)_2$ at 25 °C for 56 days	(−) Biomass C & N, C mineralization, abundance, diversity (−) Metabolism: (+) L-phenylalanine, D-mannitol, α-ketobutyric acid utilization, and (−) Tween40, pyruvic acid methyl-ester, hydroxy butyric acid, Itaconic acid utilization	Akmal et al., 2005

[a](+) or (−) indicates positive or negative correlation to lead concentration, respectively.
[b]Phylum classification was provided to each genus.

indicator of ecosystem changes or perturbations. Microbial Pb toxicity involves displacement or substitution of essential elements in nuclear proteins, inhibition of enzyme activity, and damage to cell membrane or DNA structure (Chen et al., 2018; Tipayno et al., 2018). While Pb is bacteriostatic or bactericidal to many microorganisms, some have developed resistance mechanisms that enable them to survive and even thrive. Studies comparing different levels of contamination often reveal changes in microbial community structure and function (e.g., Akmal, Wang, Wu, Xu, & Xu, 2005; Liu, Lin, Dong, Li, & Liu, 2018). Key factors influencing these changes included the magnitude and duration of the exposure as well as the ambient environment such as aquatic sediments or terrestrial soils (Azarbad et al., 2013; de Vries et al., 2007; Jaiswal & Pandey, 2018; Xu et al., 2018).

Whereas several of the studies evaluated for this review were conducted as short-term *in vitro* experiments where Pb exposure was artificially introduced by incubation with $Pb(NO_3)_2$ in a green house or laboratory setting (Table 3), most studies involved *in situ* field sampling of sites that had a contamination history of decades and longer (Tables 4 and 5). A distinction made in these *in situ* studies is between terrestrial soils (Table 4) and aquatic sediments (Table 5). In general, contaminated soils contain much higher levels of Pb and other heavy metals than aquatic sediments (Tables 4 and 5). A comparison among selected studies also suggests some broad differences in microorganisms at the phylum level between contaminated soils and sediments (Figure 2). For example, members of the phyla *Acidobacteria*, *Actinobacteria*, *Bacteriodetes*, *Proteobacteria* and *Verrucomicrobia* tend to dominate microbial communities in sites contaminated by Pb and other heavy metals (Fierer et al., 2012). While lower in relative abundance ($< 5\%$), *Chloroflexi*, *Cyanobacteria*, *Firmicutes* and *Gemmatimonadetes* generally are present. However, relative microbial community composition can vary depending on geographical location and physical/chemical properties of the soil, sediment and water. Xu et al. (2017) saw no changes in community richness or diversity between mining and control areas, however they did observe changes between geographical locations. The relative abundance of microbial communities correlated to mining contamination (Guo et al., 2018; Ren et al., 2016; Xu et al., 2017; Yin et al., 2015). Interestingly, Jacquiod et al. (2018) did observe higher microbial diversity in polluted sediments. Therefore, it is necessary to understand microbial community composition in both the contaminated and nearby uncontaminated or lessor contaminated control sites and determine if the contaminant impacts relative abundance, diversity and/or richness of the microbial community. Furthermore, Pb typically co-existed with other heavy metals in contaminated sites. Thus, most *in situ* studies on microbial communities reflect a collective effect of a group of heavy metals with Pb being a principal contaminant (Sauvé, McBride, & Hendershot, 1997). Many heavy metals had similar toxicological effects on microbial communities (Xu et al.,2019), thus the effects of Pb can be inferred from these studies. In contrast, shorter term *in vitro* Pb exposure studies uses Pb dosing and incubation to better isolate Pb-specific ecological and toxicological effects. However, their duration may not to be long enough to represent field conditions where exposure has occurred for decades and microbial communities have stabilized. Therefore, the discussion below examines early stage and long-term exposure to better parse the effects on microbial communities.

Table 4. Lead and heavy metal impact on microbial communities in terrestrial soils.

Site History	Total Metal Concentration (mg/kg)	Community Effects[a]		Reference
Industrial waste (20 years), Dapu Town, Hunan Province, China	As, 10.8–146.3 Cd, 1.09–46.55 Cr, 1.21–36.9 Pb, 33.6–2866 Zn, 57.7–1300 +Ca, Ti, V, Mn, Fe, Co, Ni, Rb, Sr, Y, Zr, Bi, Th, K	(+)[b] *Proteobacteria*, *Crenarchaeota*, *Euryarchaeota* (−) *Chloroflexi* Cd, As, Zn, Pb (+) Archea abundance; (−) Bacteria abundance		Li et al., 2017
Electronic waste & smoldering activities (Cu enrichment) Alaba Internatl Market Lagos State, Nigeria	Cd, 1.2–20.7 Cr, 16.6–187 Cu, 10–21600 Ni, 10–3830 Pb, 10–8670 Zn, 20–3040	Dominant (in descending order of abundance in high heavy metal soil): *Proteobacteria*, *Firmicutes*, *Actinobacteria*, *Chloroflexi*, *Acidobacteria*, *Planctomycetes*, *Bacteroidetes*		Jiang et al., 2019
Manufactured gas plant (150–200 years) Multiple cities, Australia	Al, 418–3341 As, 3–177 Cd, 0.04–3 Cr, 15–431 Cu, 10–1388 Ni, 6–107 Pb, 19–1622 Zn, 20–953 +Polycyclic Aromatic Hydrocarbons	Dominant: *Proteobacteria*, Gram negative bacteria Lower abundance: *Gemmatimonadetes*, *Bacteriodetes*, *Chloroflexi*		Kuppusamy et al., 2016
Au, Pb, & Zn mining, Zhen'an County, Shangluo City, Shaanxi Province, China	As, 19.83–79.81 Cd, 0.36–1.97 Hg, 0.12–2.09 Pb, 30.91–59.86 Zn, 28.63–50.68	Dominant (in descending order of abundance): *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Actinobacteria*, *Gemmatimonadetes*, *Planctomycetes*, *Firmicutes* (+) *Acidobacteria*, *Bacteroidetes*, *Planctomycetes* (−) *Proteobacteria*, *Gemmatimonadetes*, *Actinobacteria*, *Firmicutes* *Gammaroteobacteria* *Acidobacteria* *Bacteroidetes* *Alphaproteobacteria*	Genera: (+) *Acidibacter* (+) *Blastocatella* (+) *Flavobacterium*, *Pedobacter* (−) *Sphingomonas* (−) *Acinetobacter*, *Pseudomonas*, *Mizugakiibacter*, *Rhodanobacter* (−) *Gemmatimonas* (−) *Ralstonia* (−) *Arthrobacter*	Guo et al., 2017

(*continued*)

Table 4. Continued.

Site History	Total Metal Concentration (mg/kg)	Community Effects[a]		Reference
		Gammaproteobacteria *Gemmatimonadetes* *Betaproteobacteria* *Actinobacteria*		
Pb & Zn mining (1904–1970) Picher, Ottawa Co., Oklahoma, USA	Al, 34.8–4040.4 Cd, 0.1–43.6 Mg, 94.0–841.7 Pb, 3.1–1115.2 Zn, 8.1–4486.9 +Te, W, K, Mn, Ni, Fe, V, B, Cu, Co, Cr, Ti, Na	Pb, Cd, Zn, Mg (−) Bacteria Al, Cd, Pb, and/or Zn (+) or (−) *Acidobacteria*, *Actinobacteria*, *Bacteriodetes*, *Chloroflexi*, *Plantomycetes*, *Proteobacteria*, *Verrucomicrobia* *Actinobacteria* *Bacteroidetes* *Alphaproteobacteria* *Deltaproteobacteria* *Planctomycetes*	Genera: (+) *Streptomyces*, *Amycolatopsis* (+) *Flavisolibacter* (+) *Sphingomonas* (+) *Geobacter* (+) *Gemmata*, *Planctomyces*	Beattie et al., 2018 Beattie et al., 2017 (analytical chemistry)
Zn & Pb mining & smelting (since medieval times; industrial, 1967–2013) Miasteczko Śląskie & Olkusz, Poland	Cd, 3.98–82.6 Cu, 2.25–127 Mn, 57–770 Pb, 298–7200 Zn, 80–4249	(+) Gram positive bacteria (−) Gram negative bacteria & fungi		Azarbad et al., 2013
Pb & Zn enrichment facility (40 years) & Pb & Zn mining (100 years) Yunnan Province, China	Pb, 23.61–36,586 Zn, 66.99–31,089	Pb & Zn (+) *Verrucomicrobia* *Alphaproteobacteria* *Actinobacteria* *Betaproteobacteria* *Alphaproteobacteria* Mining (−) *Actinobacteria*, *Chloroflexi* Mining (+) *Verrucomicrobia* *Actinobacteria* *Alphaproteobacteria* *Betaproteobacteria* *Alphaproteobacteria*	Genera: Pb & Zn (+) *Bradyrhizobium* Pb & Zn (−) *Nocardioides*, *Gaiella*, *Acidimicrobiaceae* (Family) uncultured, *Actinobacteria* norank Pb & Zn (−) *Comamonadaceae* (Family) unclass Pb & Zn (−) *Skermanella* Mining (−) *Nocardioides*, *Gaiella*, *Acidimicrobiaceae* uncultured & *Actinobacteria norank* Mining (−) *Skermanella* Mining (−)Comamonadaceae (Family) unclass Mining (+) *Bradyrhizobium*	Xu et al., 2017
Smelter (1936–1989) Seocheon city, Chungnam, Republic of Korea	As, 1.3–16.9 Cd, 1.02–3.2 Cu, 65.33–154.8 Ni, 2.7–3.82	Dominant (in descending order of abundance): *Proteobacteria, Chloroflexi, Acidobacteria, Bacteroidetes*	Genera: As(+) Bacillus As(+) Desulfatibacillum As(−) *Desulfovirga*	Tipayno et al., 2017

(*continued*)

Table 4. Continued.

Site History	Total Metal Concentration (mg/kg)		Community Effects[a]	Reference
	Pb, 53.8–455.3	Smelting (−) *Chloroflexi, Chlorobi*	Cd (+) *Bacillus*	
	Zn, 44.6–102.6	*Firmucutes*	Cd (−) *Desulfatibacillum, Desulfoglaeba, Desulfovirga*	
		Deltaproteobacteria	Cu (+) *Streptomyces*	
		Deltaproteobacteria	Cu (−) *Desulfatibacillum, Desulfococcus, Desulfovirga*	
		Firmucutes	Pb (+) *Desulfatibacillum, Desulfovirga*	
		Deltaproteobacteria	Pb (−) *Desulfococcus*	
		Actinobacteria	Ni (+) *Desulfovirga*	
		Deltaproteobacteria		
		Deltaproteobacteria		
		Deltaproteobacteria		
		Deltaproteobacteria		

[a]Phylum unless otherwise noted. Proteobacteria are listed by class if associated information is available: Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria. When a genus is identified, its corresponding phylum is provided for clarity.
[b](+), positive correlation to heavy metal concentration; (−), negative correlation to heavy metal concentration.

Table 5. Impact of lead and other heavy metal pollution on microbial communities in aquatic sediments.

Site history	Total metal concentration (mg/kg)	Community Effects[a]		Reference
Cu Mining, Clark Fork River, Montana, USA	As, 2.01–68.9 Cd, .011–1.84 Cu, 1.14–332 Pb, 2.59–69.8 Zn, BDL[c]–433	(+)[b] *Gammaproteobacteria* (−) *Betaproteobacteria* (+) prokaryotes (−) eukaryotes & actinomycetes		Feris et al., 2003
Sewage outlet of mining and smelting operations over 30 years, Xiangjian river, Hunan Province, China	As, 73–48 Cd, 3–22.1 Co, 12.1–23.2 Cr, 57–87 Cu, 34–69 Hg, 0.18–0.65 Ni, 30.2–53.7 Mn, 788–2012 Pb, 83–124 Zn, 158–496	*Betaproteobacteria* *Gammaproteobacteria* *Firmicutes* *Firmicutes* *Deltaproteobacteria* *Alphaproteobacteria* *Betaproteobacteria* *Actinobacteria* *Bacteroidetes*	Genera: Dominant: *Janthinobacterium, Massilia* *Acinetobacter* *Proteiniclasticum* (+) *Fusibacter, Proteiniclasticum* (+) *Geobacter* (−) *Sphingomonas* (−) *Janthinobacterium* (−) *Arthrobacter* (−) *Flavobacterium*	Ren et al., 2016
Baiyin Nonferrous Metal Co., 19 million tons of wastewater discharged during 1960s–1995, Dondagou River, Gansu Province, China	As, 8.8–57.1 Cd, 0.2–5.9 Cr, 32.6–59.1 Cu, 19.7–57 Hg, BDL–1.3 Ni, 14.7–26.2 Pb, 20.1–153.7 Zn, 54.8–330.2	(+) viral abundance (+) *Proteobacteria, Cyanobacteria, Tenericutes, Firmicutes, Bacteroidetes* (−) *Verrucomicrobia, Actinobacteria, Chloroflexi* *Betaproteobacteria* *Gammaproteobacteria* *Bacteroidetes* *Planctomycetes*	Genera: (+) *Thauera, Hydrogenophaga, Thiobacillus, Acidovorax, Albidiferax, Ramlibacter, Methyloversatilis* (+) *Luteibacter* (+) *Algoriphagus* (+) *Pirellula*	Chen et al., 2018
MetalEurop Smelter, over 100 years of accidental discharges into river from 1893, River Deûle, France	MetalEurop Cd, 1.29–38.13 Cu, 13.76–99.99 Pb, 11.58–913.83 Zn, 348.6–3218.3 +Al, As, Co, Cr, Fe, Mn, Ni, V	*Gammaproteobacteria*	Genus: Pb, As, Cd, Co, Cr, Cu, Ni, and Zn (+) *Pseudomonas*	Roosa, Wattiez, et al., 2014; Roosa, Wauven, et al., 2014
MetalEurop Smelter, Decades of accidental discharges into river from 1893, River Deûle, France Férin, Sansée Canal, France	MetalEurop Cd, 38.13 Cu, 99.99 Pb, 913.83 Zn, 3218.27 +Al, As, Co, Cr, Fe, Mn, Ni, V	*Betaproteobacteria* *Gammaproteobacteria* *Alphaproteobacteria* *Deltaproteobacteria* Gammaproteobacteria *Betaproteobacteria*	Dominant: *Burkholderia, Rubrivivax, Leptothrix, Cupriavidus, Methylibium, Variovorax, Thauera, Azoarcus* *Pseudomonas* *Methylobacterium*	Gillan et al., 2015

(*continued*)

Table 5. Continued.

Site history	Total metal concentration (mg/kg)	Community Effects[a]		Reference
	Férin Cd, 1.29 Cu, 13.76 Pb, 111.58 Zn, 348.55 +Al, As, Co, Cr, Fe, Mn, Ni, V	*Betaproteobacteria* *Deltaproteobacteria* *Actinobacteria*	*Anaeromyxobacter* Genera: (+) *Pseudomonas, Pseudoxanthomonas, Stenotrophomonas* (+) *Thiobacillus, Acidovorax, Dechloromonas, Alicycliphilus* (−) *Rubrivivax, Leptothrix* (−) *Anaeromyxobacter, Sorangium* (−) *Mycobacterium, Streptomyces*	
MetalEurop Smelter, Decades of accidental discharges into river from 1893, River Deûle, France Férin, Sansée canal, France	MetalEurop Cd, 38.13 Cu, 99.99 Pb, 913.83 Zn, 3218.27 +Al, As, Co, Cr, Fe, Mn, Ni, V Férin Cd, 1.29 Cu, 13.76 Pb, 111.58 Zn, 348.55 +Al, As, Co, Cr, Fe, Mn, Ni, V	(−) *Gammaroteobacteria* (−) *Firmicutes* (−) *Alphaproteobacteria* (with higher RNA levels (more active) (+) *Actinobacteria*, with lower RNA levels (sensitivity)	Orders: *Enterobacteriales*, *Pseudomonadales*, Aeromonadales dominant) *Clostridiales*, *Lactobacillales*	Jacquiod et al., 2018
Poyang Lake, China Tungsten & other mining	As, 4.23–10.95 Cd, 0.307–1.022 Pb, 29.40–40.06 +Fe, Li, Cr, Co, Ni, Cu, Zn, W, Tl, Sn, Sb	(−) *Betaproteobacteria*, *Gammaproteobacteria* (+) *Acidobacteria, Latescibacteria, Verrucomicrobia, Nitrospirae, Gemmatimonadetes*		Zhang, Xu, et al., 2018; Zhang, Wan, et al., 2018
Heavy metal spill in April 2016, Xiannvhu Lake, Jiangxi Province, China	As, 098–137.87 Cd, 0.13–137.87 Cu, 5.31–119.32 Pb, 3.75–54.12 Zn, 14.44–205.59	(−) Denitrifiers *Betaproteobacteria* *Gammaproteobacteria*	Genera: Primary denitrifiers: *Pseudogulbenkiania* *Pseudomonas* + 2 unknown groups	Guo et al., 2018
Zn & Ti smelters (80 years) Sørfjord, southern Norway	Cd, 0.22–3.76 Cu, 1.32–43.83 Pb, 12.18–259.71 Zn, 18.72–333.37	Cu, Pb, Zn (−) *Gammaproteobacteria*, *Cytophaga-Flexibacter-Bacteroides* Group (*Bacteroidetes*) No correlation with *Deltaproteobacteria*		Gillan et al., 2005

[a]Phylum unless otherwise noted. Proteobacteria are listed by class if associated information is available: Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, and Gammaproteobacteria. When a genus is identified, its corresponding phylum is provided for clarity.
[b](+), positive correlation to heavy metal concentration; (−), negative correlation to heavy metal concentration.
cBDL, Below detection limit.

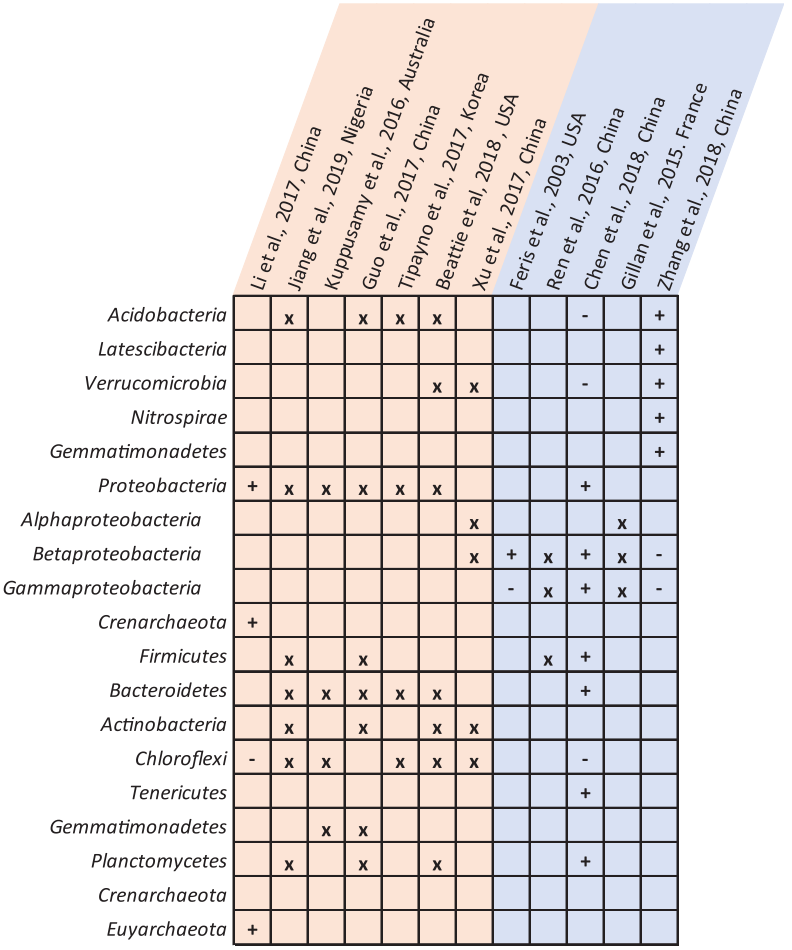

Figure 2. Bacterial phyla in selected in situ studies of contaminated soils (light brown) and aquatic sediments (light blue): x denotes dominant and + or − indicates positive or negative correlation with levels of Pb and other heavy metals.

3.1. Microbial communities under early stage exposure to lead contamination

Several studies have indicated changes in microbial communities even at early stages of exposure, including suppressed microbial metabolism, reduced biomass production in association with higher energy demand, changes in diversity and relative abundance, and microbial community shifts (Table 3; Akmal et al., 2005; Liu et al., 2018; Sobolev & Begonia, 2008; Xu et al., 2018). For example, Akmal et al. (2005) amended soil with $Pb(NO_3)_2$ and found that utilization of L-phenylalanine, D-mannitol, α-ketobutyric acid increased while Tween40, pyruvic acid methyl-ester, hydroxy butyric acid, itaconic acid utilization decreased. These results indicate an alteration in microbial community-level metabolism and activity (Akmal et al., 2005). Other immediate inhibitory

effects of Pb are reflected in C and nutrient cycling such as effects on the denitrifying microbial community (Sobolev & Begonia, 2008; Xu et al., 2019). Sediments from a lentic system acutely contaminated by a heavy metal spill (Cd, Zn, Pb, Cu, As) showed reduced abundance of the nitrous oxide reductase gene, *nos*Z, suggesting lower denitrifier community richness (Table 4; Guo et al., 2018). Following the spill, the dominant *nos*Z-denitrifier genus was *Pseudogulbenkiania* (*Betaproteobacteria*), whereas the genus *Pseudomonas* (*Gammaproteobacteria*) increased following the spill and two unidentified denitrifier groups decreased (Guo et al., 2018). This indicated that some members of the microbial community reacted to contamination of Pb and other heavy metals, in part, by developing metal-resistant mechanisms while others remained susceptible. The selection of microorganisms for Pb-resistant forms of nitrite reductase in early stages of exposure to Pb also was evidenced by changes in the community harboring *nir*K in $Pb(NO_3)_2$-amended soil (Sobolev & Begonia, 2008). Their study further noted that Pb had detectable effects on community diversity at relatively low concentrations, and that several concentration thresholds were observed for shifts in community diversity.

The impact of Pb exposure on microbial community structure changes with Pb availability. Soil incubated with $Pb(NO_3)_2$ (5000 mg/kg for 49 days) resulted in an increase of Gram positive bacteria accompanied by a decrease in the abundance of fungi and actinomycetes (Xu et al., 2018). The relative abundance of Gram negative bacteria was unaffected based on their increased numbers in the Pb spiked soil. Upon the addition of macadamia nutshell biochar, which reduces Pb bioavailability, fungi and actinomycetes recovered slightly and microbial respiration and biomass production increased (Xu et al., 2018). When Pb-amended soil was acclimated for four weeks, the dominant phyla observed were *Bacteroidetes, Proteobacteria, Firmicutes,* and *Actinobacteria*. *Acidobacteria, Verrucomicrobia,* and *Chloroflexi* were less abundant. However, as the Pb concentration decreased during phytoremediation, the latter phyla increased in abundance. At the genus level, *Bacillus, Adhaeribacter, Pontibacter, Flavisolibacter*, and *Kaistobacter* were present in the original Pb-amended soil. Following phytoremediation, *Flavisolibacter, Kaistobacter,* and *Pseudomonas* increased in abundance with a relative decrease in *Bacillus, Adhaeribacter, Pontibacter*, and *Paenibacillus*, thus suggesting reduced Pb tolerance. Controlled studies clearly show that Pb bioavailability and bioremediation influence the community structure in Pb-amended soil (Liu et al., 2018; Xu et al., 2018).

3.2. Microbial communities under long-term exposure to lead and other heavy metal contamination: terrestrial soils

Lead and Zn mining and Pb-related industrial activities have occurred globally for decades and left an environmental footprint as contaminated soils

that influence microbial biomass, activity, and diversity (Table 4; Azarbad et al., 2013; Beattie et al., 2018; Chen et al., 2014; Guo, Kang, & Feng, 2017; Xu et al., 2017), confirming the results of short term exposure studies summarized above. Biomass and respiration were negatively correlated with levels of nine metals including Pb, accounting for differences in microbial community structure (Poland, USA; Azarbad et al., 2013; Beattie et al., 2018). At a Pb battery recycling facility that contaminated soil for 40 years (Pb up to 10,000 mg/kg; 5% water), microbial biomass and respiration were apparently decreased. However, addition of a C source stimulated biomass production (Shi et al., 2002), partly due to reduced Pb bioavailability by the presence of organic matter (Azarbad et al., 2013). Near a metallurgy plant (in operation since the 1970s), Pb, Cd, Cu and Zn contaminated-soil also reduced microbial biomass and respiration as well as dehydrogenase activity (Chen et al., 2014). However, conflicting reports exist in the literature on the impact of Pb and other heavy metals on soil microbial community diversity. For example, twenty years of heavy metal contamination by Pb and Cd explained the dissimilarity of microbial community diversity and richness between contaminated and uncontaminated soils (China; Hu, Qi, Zeng, & Zhang, 2007). At the site of a large mining operation which ceased in the mid-1950s (USA), bacterial abundance was negatively correlated with Pb, Cd, Zn, Mg whereas Archaea were positively correlated with pH (Beattie et al., 2018). Gram positive bacteria positively correlated to heavy metal contamination and Gram negative bacteria and fungi showed a negative correlation at a Zn and Pb mining/smelting site in Poland (Azarbad et al., 2013). However, at a site with over 100 years of mining operations and 40 years of Pb and Zn enrichment (up to 30,000 mg/kg), no significant heavy metal induced differences in diversity or richness indices were detected between mined and unmined locations (China; Xu et al., 2017), suggesting that the bacterial community in abandoned mines tends to stabilize and increase its diversity. Similarly, in soils contaminated for twenty years by industrial waste, microbial diversity richness and evenness were not statistically linked to heavy metal concentration (Li et al., 2017). Other factors such as soil chemical properties including organic matter contents and pH influenced microbial diversity more than heavy metal contents (Jiang et al., 2019).

Lead and other heavy metals are statistically linked to changes in microbial community structure in heavy metal contaminated soils (Azarbad et al., 2013; Beattie et al., 2018; Guo et al., 2017; Xu et al., 2017). In a site contaminated for twenty years by industrial waste, Archea abundance was positively correlated to Cd, As, Zn and Pb, and Bacteria were negatively correlated to these metals (Li et al., 2017). *Proteobacter, Crenarchaeota* and *Euryarchaeota* were more abundant in the heavy metal contaminated soil

whereas *Chloroflexi* were more abundant in uncontaminated soil (Li et al., 2017). Electronic waste and Cu smelting activities contaminated surrounding soil with Pb as well as Cu, Zn, Cd, Cr and Ni where *Proteobacteria, Firmicutes, Actinobacteria, Chloroflexi, Acidobacteria, Planctomcetes* and *Bacteroidetes* dominated in descending order (Jiang et al., 2019). Manufactured gas plants (Australia), reportedly 150-200 years old, resulted in polycyclic aromatic hydrocarbon and heavy metal (Cr, Ni, Cu, Zn, As, Cd, Pb) soil contamination which selected for *Proteobacteria* and other Gram-negative bacteria, *Gemmatimonadetes* and *Bacteriodetes*, and influenced *Chloroflexi,* which were present in lower abundance (Kuppusamy et al., 2016). At the site of a large mining operation which ceased in the mid-1950s (USA), Cd, Pb, and Zn contamination significantly influenced on selection of the phyla *Acidobacteria, Actinobacteria, Bacteriodetes, Chloroflexi, Plantomycetes, Proteobacteria* and *Verrucomicrobia* in the soil microbial community (Beattie et al., 2018). The phyla *Proteobacteria, Acidobacteria, Bacteroidetes, Actinobacteria, Gemmatimonadetes Planctomycetes* and *Firmicutes* (in descending order of dominance) were associated heavy metal contamination in soils polluted by mining wastes in China (Guo et al., 2017). In another mining site in China, *Actinobacteria* and *Chloroflexi* were more dominant in the mine soil compared to unmined soil where *Verrucomicrobia* were more abundant (Xu et al., 2017). These changes in microbial community structure clearly show the selection of tolerant groups under long-term exposure to heavy metal contamination (mostly belonging to the class *Proteobacteria*) while sensitive ones are reduced.

The specific effects on microbial community structure can be better elucidated at lower taxonomic levels when the phylum level is inadequate to detect the differences. Guo et al., 2017 showed that at the genus level, *Sphinogomonas* was the most abundant genus in soils polluted by mining wastes in China, and *Acinetobacter, Pseudomonas, Gemmatimonas, Ralstonia, Mizugakiibacter, Rhodanobacter, Arthrobacter, Acidobacter, Blastocatella, Flavobacterium* and *Pedobacter* correlated with Cd and Pb levels ($p < .05$). *Ralstonia* and *Gemmatimonas* negatively correlated with Cd, Pb, As, Hg and pH; *Mizugakiibacter* and *Rhodanobacter* negatively correlated with Cd, Pb, As and soluble organic matter; and *Blastocatella*, an unidentified *Nitrospiraceae*, and an unidentified *Acidobacteria* positively correlated with Pb, Cd, Zn, Hg and soluble organic matter (Guo et al., 2017). At a Pb and Zn enrichment site with many years of mining operation in China, Xu et al. (2017) reported that metal concentrations negatively correlated with *Actinobacteria* (class level), *Comamonadaceae* and *Acidimicrobiaceae* (family level), and *Nocardioides*, *Gaiella*, and *Skermanella* (genus level). These microorganisms also had less representation in uncontaminated samples. In contrast, abundances of *Verrucomicrobia* and

Bradyrhizobium showed positive correlations with Pb and Zn levels. At another site contaminated by Pb and other heavy metals (As, Cd, Cu, Ni, and Zn) with approximately fifty years of nonferrous smelter operation (ceased in 1989, South Korea), Tipayno et al. (2018) indicated that variations in the bacterial community structure were mostly related to general soil properties such as soil pH at the phylum level, while at the finer taxonomic levels, the concentrations of As and Pb were the significant factors. For example, at the genus level, Pb positively correlated with *Desulfatibacillum* and *Desulfovirga*; and negatively correlated with *Desulfococcus* (Tipayno et al., 2018).

Advanced molecular analyses, such as polymerase chain reaction (PCR) amplification and sequencing, may inform the influence of Pb and other heavy metals on community functional properties in addition to structural changes. Tipayno et al. (2018) conducted analysis of community functional profiles ("pathway abundance profiles") and they indicated that an increase in genes encoding enzymes associated with DNA replication and repair, translation, transcription, and nucleotide metabolism pathways in metal contaminated soil. In contrast, genes encoding enzymes associated with amino acid, lipid, and energy metabolism and biodegradation potential of xenobiotics were less abundant (Tipayno et al., 2018). In other words, levels of As, Cd and Pb were positively correlated with enzymes associated with cell growth/death, transcription, signaling molecules, and interaction pathways, and negatively correlated enzymes associated with transport, catabolism and metabolism of terpenoids and polyketides. Epelde, Lanzén, Blanco, Urich, and Garbisu (2015), using a metatranscriptomic approach, also indicated that genes involved in transposition and transfer of genetic material were significantly more abundant in highly contaminated soils from an abandoned Pb-Zn mine site whereas genes related to stress and starvation responses were more abundant in less contaminated samples. Clearly, industrial Pb and other heavy metals contaminating soil for decades have shaped the resident microbial community structure and are linked to their functional differences, regardless of geographical region.

3.3. Microbial communities under long-term exposure to lead and other heavy metal contamination: aquatic sediments

Sediment microbial communities in aquatic systems adjacent to mining and smelting operations are impacted by heavy metal contamination, with Pb identified as one of the primary constituents (Table 5; Feris et al., 2003; Gillan, Danis, Pernet, Joly, & Dubois, 2005; Jie et al., 2016; Roosa, Wattiez, et al., 2014; Roosa, Wauven, et al., 2014; Zhang, Xu, et al., 2018; Zhang,

Wan, et al., 2018). In sediments contaminated by Cu (USA) and tungsten (W, China) mining operations, which contain significant quantities of Pb, dissimilarity in microbial communities increased along an increasing chemical concentration gradient (Feris et al., 2003; Zhang, Xu, et al., 2018; Zhang, Wan, et al., 2018). However, in sediments contaminated by Cu mining activity, biomass was unaffected by metal concentration with no apparent correlation between sediment metal content and diversity or total productivity (Feris et al., 2003), similar to some of the soil studies (e.g., Xu et al., 2017). This implies that other factors controlling biomass override any impacts of heavy metal contamination. The structure of microbial communities, however, was significantly affected. Phospholipid fatty acid analysis (PLFA) indicated that heavy metal concentration was positively correlated with prokaryote abundance but was negatively correlated with eukaryotes and actinomycetes. Further analysis by quantitative polymerase chain reaction (qPCR) using specific primers revealed a positive correlation of metal contamination with *Gammaproteobacteria* and a negative correlation with *Betaproteobacteria* abundance (Feris et al., 2003). At the W mining site in China, 16S rRNA gene sequencing identified *Proteobacteria* and *Actinobacteria* as the dominant sediment phyla (Zhang, Xu, et al., 2018; Zhang, Wan, et al., 2018). While all metal-contaminated sites had a unique microbial community composition, they also shared many members in common. Zhang, Xu, et al. (2018) and Zhang, Wan, et al. (2018) confirmed the negative correlation of metal concentration with *Betaproteobacteria* and *Deltaproteobacteria* while members of the phyla *Acidobacteria*, *Latescibacteria*, *Verrucomicrobia*, *Nitrospirae* and *Gemmatimonadetes* were positively correlated with metal concentration.

Sediments sampled along a gradient from a sewage outfall on the Xiangjian River (China), linked to heavy non-ferrous metal mining and smelting operations over the past 30 years, have been studied extensively to understand the effects of heavy metal stressors on both the microbial communities and functional genes (Table 5). Inductively coupled plasma atomic emission spectroscopy revealed that the sediments were contaminated with Cu, Pb, Zn, As, Cd, Ni, Hg, Cr, Mn, and Co, forming a gradient of decreasing concentrations with distance from the sewage outlet (Jie et al., 2016; Ren et al., 2016; Yin et al., 2015). Microbial communities in sediments closest to the outfall demonstrated lower Shannon diversity values and different Pielou eveness indices relative to other less contaminated sediments (Ren et al., 2016; Yin et al., 2015). Members of the phyla *Firmicutes*, *Chloroflexi* and *Crenarchaeota* were found more abundant in the highly contaminated sediments whereas *Proteobacteria* and *Actinobacteria* were present but in lower abundance (Yin et al., 2015). Molecular ecological network analysis revealed that Pb, as well as mercury

(Hg), Zn and C, correlated with the network module containing the phyla *Bacteroidetes, Chloroflexi, Proteobacteria, Acidobacteria, Firmicutes* and *Actinobacteria* from the highly contaminated sediments, leading Yin et al. (2015) to suggest that microbial community composition and co-occurrence of phyla are driven by heavy metals in the sediment. Dominant microbial genera in all sediments along the gradient were *Fusibacter*, *Janthinobacterium*, *Proteiniclasticum*, *Acinetobacter*, and *Massilia* with ~15-18% unclassified to the genus level. *Fusibacter*, *Geobacter*, and *Proteiniclasticum* were more abundant in sediments with higher heavy metal concentration whereas *Janthinobacterium*, *Arthrobacter*, *Sphingomonas* and *Flavobacterium* were more abundant in less contaminated samples (Ren et al., 2016). Each sediment sample also had a unique microbial community functional gene structure though all samples along the gradient were ~80-90% similar. Whole microbial community functional gene structure and Pb resistant genes abundance correlated with Pb concentration across the gradient (Jie et al., 2016). In the most heavily contaminated sediment, genes encoding for heavy metal resistance and C cycling pathways, including degradation of aromatic and nitroaromatic compounds, were more prevalent (Yin et al., 2015).

The Dondagou River (China), a tributary to the Yellow River, is heavily contaminated with Cu, Zn, Cd, Pb, As, Hg, Cr and Ni due to approximately 19 million tons of wastewater discharge from non-ferrous metal mining and processing during the 1960s – 1995 (Table 5; Li, Wang, Gou, Su, & Wang, 2006; Li, Yao, Tian, Xu, & Li, 2006; Chen et al., 2018). Lead concentrations have been recorded in the same range as those observed in the Xiangjian River site (153 mg/kg vs 124 mg/kg), with other heavy metals present at comparable levels at both sites (Chen et al., 2018; Ren et al., 2016). Microbial community diversity was found negatively correlated with heavy metal concentration and carried a higher viral load than sediments from less contaminated sites. The phyla *Proteobacteria*, *Cyanobacteria*, *Tenericutes*, *Firmicutes*, and *Bacteroidetes* abundance showed a positive correlation with heavy metal contamination while *Verrucomicrobia*, *Actinobacteria* and *Chloroflexi* were less represented (Chen et al., 2018). *Proteobacteria, Bacteroidetes* and *Firmicutes* were the most dominant phyla harboring heavy metal resistance and reduction genes, however, heavy metal resistance and reduction genes also were detected in the Bacteria *Gemmatimonadetes*, *Planctomycetes, Acidobacteria, Tenericutes, Spirochetes, Chlorobi* and *Parubacteria* and the Archea *Euryarchaeota* and *Thaumarchaeota* using qPCR analysis. Within the phylum *Proteobacteria*, the classes *Betaproteobacteria*, *Gammaproteobacteria*, and *Alphaproteobacteria* harbored the highest number of heavy metal resistance genes, which also were present in a few representatives of the classes *Deltaproteobacteria* and

Zetaproteobacteria (Chen et al., 2018). Genes associated with DNA recombination, DNA damage repair, and heavy metal resistance were more prevalent in the more highly contaminated sediments, supporting the findings of Epelde et al. (2015) and Tipayno et al. (2018) with contaminated terrestrial soils.

In northern France, accidental discharges of Cd, Cu, Pb, and Zn from a smelter located on the River Deûle occurred over a 100 yr period (Table 5; Roosa, Wattiez, et al., 2014; Roosa, Wauven, et al., 2014). Directly adjacent to the smelter, sediment Pb concentration was found to average 913 mg/kg and co-occurred with Al, As, Cd, Co, Cr, Cu, iron (Fe), manganese (Mn), Ni, vanadium (V), and Zn (Gillan, Roosa, Kunath, Billon, & Wattiez, 2015). Lead concentrations were higher than in the most contaminated sediments from the Xiangjian River (737 vs 913 mg/kg; Jie et al., 2016). The upstream Pb concentrations averaged 112 mg/kg, comparable to those observed in two of the Xiangjian River studies (Chen et al., 2018; Ren et al., 2016). Sediments from River Deûle and less contaminated sites upstream have been studied extensively to elucidate the impact of heavy metal contamination on microbial community structure and function and to better understand how these communities adapt to the presence of heavy metals (Gillan et al., 2015; Jacquiod et al., 2018; Roosa, Wattiez, et al., 2014; Roosa, Wauven, et al., 2014). At the phylum level, microbial communities in sediments adjacent to the smelter were found to be similar to those in the upstream less contaminated sites, possibly reflecting the effect of long-term exposure to contamination. This finding is comparable to results from a study of marine sediment contaminated for 80 years by a Zn and Pb smelter operations (Norway), which reported that microbial communities at contaminated and uncontaminated sites were similarly diverse due to acclimation over the 80-year period (Gillan et al., 2005). Also note that there is a potential for microorganisms in upstream sediment to continually re-inoculate sediment downstream in a riverine system. If these microbes are resistant to heavy metals or can acquire resistance, they have the potential to become members of the more highly contaminated sediments.

It is noteworthy that while the metal contaminated site and upstream sediments contained taxonomically similar microbial communities (70% similar), functionally they were different (Gillan et al., 2015). The higher-Pb sediments contained more genes encoding "cell wall and capsule substances", "virulence, disease and defense mechanisms", and "phages, prophages, transposable elements and plasmids" (Gillan et al., 2015). For example, Co/Zn/Cd efflux system genes, *czc*A and *czc*D, and genes encoding exopolysaccharides were more prevalent in the higher-Pb sediments (Gillan et al., 2015). The change in the community genetic potential

clearly showed a selective advantage for Pb resistance in the contaminated sediment. *Betaproteobacteria* dominated at both Pb contaminated sites, primarily by the genera *Burkholderia, Rubrivivax, Leptothrix, and Cupriavidus. Gammaproteobacteria* (*Pseudomonas*) and *Alphaproteobacteria* (*Methylobacterium*) also were represented. *Pseudomonas, Thiobacillus, Acidovorax, Dechloromonas, Pseudoxanthomonas, Stenotrophomonas and Alicycliphilus* were more prevalent in the higher contaminated sediment whereas *Rubrivivax, Anaeromyxobacter, Leptothrix, Sorangium, Mycobacterium and Streptomyces* were more numerous in the less contaminated, upstream site (Gillan et al., 2015; Shi et al., 2002).

Roosa, Wattiez, et al. (2014) and Roosa, Wauven, et al. (2014) further noted that metal-contaminated sediments contain microorganisms from the phyla *Actinobacteria (Mycobacterium vaccaevaccae, Mycobacterium llatzerense, Rhodococcus erythreus, Streptomyces coelicoflavus), Alphaproteobacteria (Sphingomonas xenophaga, Methylobacterium extorquens, Methylobacterium radiotolerans), Gammaproteobacteria (Klebsiella oxytoca, Klebsiella ornithinolytica, Enterobacter minipressuralis, Enterobacter mori, Pseudomonas nitroreducens, Pseudomonas monteilli, Pseudomonas putida, Pseudomonas lutea, Pseudomonas putida, Pseudomonas arsenicoxydans, Aeromonas salmonicida)* and *Betaproteobacteria (Delftia lacustris)*, all of which were resistant to Pb and/or other heavy metals. The *Pseudomonas* community was positively correlated with Pb as well as Cu, Co, Ni, and Zn, as evidenced by 16S rRNA and qPCR detection of the outer membrane lipoprotein I (*opr*I) gene, specific for *Pseudomonas* spp. The *pbr*T gene (Pb(II) uptake permease protein) was present in the high-Pb sediments, however correlation with Pb concentration was inconclusive (Roosa, Wattiez, et al., 2014; Roosa, Wauven, et al., 2014). Interestingly, copy numbers of the *czc*A gene (encodes Co/Zn/Cd efflux pump) showed a positive correlation with Pb concentration even though it was not a *czc*A target metal.

Taking a more precise 16S rRNA gene DNA and 16S rRNA (measure cDNA) sequencing approach, Jacquiod et al. (2018) confirmed that the microbial community was dominated by *Proteobacteria* with *Firmicutes* the second most prevalent in the River Deûle foundry contaminated sediments. *Alphaproteobacteria* and *Betaproteobacteria* were more numerous in metal contaminated sediments whereas *Gammaproteobacteria* were more abundant in less-contaminated sediments. *Alphaproteobacteria* 16S rRNA levels (cDNA) relative to 16S rRNA gene (DNA) levels (RNA/DNA) were higher in metal contaminated sediment, implying increased activity; *Actinobacteria* had a lower RNA/DNA ratio, suggesting sensitivity. The *Bacteroidetes* represented the passive part of the community. *Ignavibacteriae*, *Deltaproterobacteria*, *Gemmatinmonadetes* and *Verrucomicrobia* were present in low abundance in the metal contaminated sediments. However, the

three latter groups, as well as *Acidobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Nitrospirae*, *Planctomycetes*, had increased RNA/DNA ratios (Jacquiod et al., 2018). The Functional Response Group (FRG) analysis employed in this study revealed that the metal contaminated sediments contained "seed bank" (metal tolerant/slow growing or inactive, e.g. *Gammaproteobacteria*, *Betaproteobacteria*, *Bacteroidetes*), "upcoming bacteria" (metal tolerant, active or passive, e.g. *Firmicutes*, *Proteobacteria*), "fecal-related bacteria" (e.g. *Clostridium*, *Enterobactericeae*), "dominant metal sensitive bacteria" (lower DNA and RNA signal, e.g. *Gammaproteobacteria*, *Pseudomonas*) and "rare metal sensitive bacteria" (significantly impacted by metals, e.g. *Bacteriodetes*, *Acidobacteria*, and *Deltaproteobacteria*) (Jacquiod et al., 2018). This elegant analysis confirms that metal resistance mechanisms and adaptation are at work in these long-term contaminated sediments.

4. Leveraging lead resistance genes for lead biosensor development

Enhanced understanding of Pb resistance genes involved in uptake, efflux, and accumulation of Pb(II) has allowed for development of several Pb biosensors based on selective regulatory proteins, DNAzymes, and whole-cell microbial bioreporters (Zhang et al., 2017). These biosensors can produce a quantifiable output in response to Pb levels in the environment. While real world applications of Pb biosensors have remained limited, Pb biosensors have great potential for use in ecological risk assessment as an effective tool for monitoring and quantifying bioavailable Pb in environmental samples. This review focuses on the understanding of Pb resistance genes for biosensor development and the accuracy, specificity and sensitivity of available Pb biosensors.

4.1. Lead resistance genes

Metal resistance genes are chromosomally- and plasmid-linked (Janssen et al., 2010) as being indicated in the Pb resistant *C. metallidurans* strain CH34 (Borremans et al., 2001; Hynninen et al., 2010; Mergeay et al., 1985). The functional aspects of the Pb resistance operon, *pbrUTRABCD*, have been elegantly described (Borremans et al., 2001; Chen et al., 2007; Hynninen et al., 2009; Monchy et al., 2007) and are elucidated below. The operon confers uptake, efflux and accumulation of Pb(II). The Pb(II) uptake permease protein, *pbrT*, transports Pb(II) into the cell (Borremans et al., 2001; Jencova et al., 2008). Pb(II) binds to the *MerR* family *pbrR* regulator, which, in turn, induces transcription of *pbrABCD* from the *pbrA* promoter (Borremans et al., 2001; Hobman et al., 2012). An intracellular Pb chaperone protein (Taghavi et al., 2009), the *pbrD* gene product, binds

Pb and transfers it to the cell membrane where the *pbrA* gene product, a P_{1B}-type ATPase efflux protein actively exports Pb(II) to the periplasm (Rensing et al., 1998). The *pbrB* gene product, an undecaprenyl pyrophosphate phosphatase (C_{55}-PP phosphatase), produces inorganic phosphate (from the cell membrane) which combines with Pb(II) to form Pb phosphate and is sequestered in the periplasm. This leads to downregulation of the *pbr* operon; *pbrC* and *pbrD* gene product synthesis is initiated (Hynninen et al., 2009). The *pbrD* gene product also may accumulate Pb(II) and prevent increased uptake of Pb(II) into the cell (Taghavi et al., 2009). The *pbr*C gene product is a lipoprotein signal peptidase that interacts with *pbrB* gene product (Taghavi et al., 2009). The *pbrU* gene product may be a major facilitator superfamily (MFS) membrane bound permease; *pbrU* is inactivated in *C. metallidurans* (Taghavi et al., 2009; Van Houdt, Monchy, Leys, & Mergeay, 2009). Both *pbrA* and *pbrB* are required for Pb resistance; *pbrT*, *pbrC*, *pbrD* and *pbrU* are not (Hynninen et al., 2009; Taghavi et al., 2009). Knowing gene function and gene interaction with Pb can inform Pb biosensor development to improve both sensitivity and specificity.

C. metallidurans strain CH34 harbors additional genes that confer Pb resistance. The *pbr*R_2 (Rmet_2302), *cadA*, *pbrC*$_2$ operon is located on chromosome 1 in a genomic island (CMGI-1) and may maintain low cellular Pb(II) concentration (Taghavi et al., 2009). The *zntA* gene (Zn(II) efflux protein), which is induced by Pb(II) (a *Staphylococcus aureus* CadA (Cd(II) efflux protein homolog in *Escherichia coli* induced by Zn(II)/Cd(II)/Pb(II) (Beard et al., 1997; Rensing et al., 1998), is a P-Type ATPase located on chromosome 2. The *pbrR*$_3$ gene (Rmet_3456; pbrR691) which preferentially binds Pb(II), is located on chromosome 1 (Monsieurs et al., 2011; Taghavi et al., 2009). These genes have been shown to rescue mutations in complementary genes in the *C. metallidurans* strain CH34 primary Pb resistance operon, *pbrUTRABCD* (Taghavi et al., 2009). Because the prevalence of host Pb resistance genes, which have the potential to bind Pb(II) and interfere with biosensor Pb detection, minimizing their presence should improve detection.

Like the *pbrR*$_2$, *cad*A, and *pbr*C_2 operons (Rmet_2302), Pb resistance genes have been shown to be associated with genomic islands flanked by mobile genetic elements (Monchy et al., 2007; Taghavi et al., 2009; Van Houdt et al., 2009). For example, in *Cupriavidus metallidurans*, Pb(II) induced genes involved in transposition (*tnp*A, *tnp*R, and *orf*-2) and open reading frames (ORFs) from truncated insertion sequence (IS) elements (*orf*-102 and *orf*-103). Furthermore, the Pb resistance *pbr* operon was found to be flanked by TN*4380*, a mercury transposon, suggesting potential for mobilization in the presence of Pb (Monchy et al., 2007). *Delftia acidovorans* strain SPH-1 was found to harbor *pbr* genes on a chromosomally-

Table 6. Examples of multiantibiotic and multiheavy metal resistance in bacteria.

Source	Resistance	Phylum	Species	Reference
Industrial waste water	14 antibiotics & 5 heavy metals	*Gammaproteobacteria*	*Acinetobacter baumanni*	El-Sayed, 2016
Surface water	15 antibiotics & 11 heavy metals	*Gammaproteobacteria*	*Raoultella planticola*	Koc et al., 2013
Soil	3 antibiotics & 7 heavy metals[b]	*Actinobacteria*	*Microbacterium* sp.	
Coast	16 antibiotics & 5 heavy metals	–	Gram negative bacteria	Matyar, 2012
River	4 antibiotics & 5 heavy metals	–	Gram negative bacteria	Matyar et al., 2014

linked genomic island with other metal resistance determinants (Van Houdt et al., 2009). Resistance ascribed to plasmid pMOL30 also conveys resistance to Ag(I), Cd(II), Co(II), Hg(II), and Zn(II) (Mergeay et al., 1985; Monchy et al., 2007). Interestingly, antibiotic resistance, which also can be linked to mobile genetic elements, was reported to co-occur with heavy metal resistance (including Pb) in many Bacteria (Bharagava, Yadav, & Chandra, 2014; El-Sayed, 2016; Hu & Chen, 2016; Koc, Kabatas, & Icgen, 2013; Matyar, 2012; Matyar et al., 2014; Pal et al., 2017; Pirela, Suárez, & Vargas, 2014; Seiler & Berendonk, 2012; Tomova, Stoilova-Disheva, Lazarkevich, & Vasileva-Tnkova, 2015). Antimicrobial resistance genes showed correlation to heavy metal contamination in sediments (Ohore, Addo, Zhang, Han, & Anim-Larbi, 2018). Table 6 shows examples of multi-antibiotic & multiheavy metal resistance bacteria such as *Bacillus subtilis*, *Bacillus cereus*, *Bacillus pumilus*, *Frankia* sp., *Acinetobacter baumanni*, *Raoultella planticola*, *Microbacterium* sp., *Vibrio parahaemolyticus, Burkholderia sordidcola*, and *Pantoea* sp. The fact that these bacteria are resistant to both antibiotics and heavy metals suggests that they have the ability to accumulate a suite of resistance genes. Each of these genes may encode a unique resistance functionality to a particular antibiotic or heavy metal and some of them may possess similar resistance mechanisms, such as efflux pumps, that provide resistance to several pollutants. Both antibiotic and heavy metal resistance can be leveraged to isolate strains that are useful indicators of Pb and other heavy metal bioavailability.

4.2. Lead biosensors

Biological sensors are biologically active organisms that can detect the substrate, transport it into the cell or bind it on the cell surface through resistance mechanisms, and produce a rapid, easy to measure response. Biosensors have been developed to detect Pb by incorporating Pb resistance genes, such as *pbrR* and *pbrA* and the luciferase reporter (*luxCDABE*) or

red fluorescence protein (RFP) into a bacterial strain, such as *Cupriavidus metallidurans* AE2448 (formally *Alcaligenes eutrophus*; also referred to as BIOMET® Pb Biosensor or strain AE2450) or Escherichia coli strain DH10B (Table 7; Geebelen et al., 2003; Wei et al., 2014; Van Der Lelie, Verschaeve, Regniers, & Corbisier, 2000). While Pb specific biosensors are rare, *C. metallidurans* AE2448, which detects 0.5 µM Pb, did not detect any other heavy metals tested (Zn, Cu, Cd, Hg, Bi, Tl, Au; Corbisier et al., 1999). Lead specific BIOMET® (*Cupriavidus metallidurans* AE2448/AE2450) successfully detected 7 – 404 mg/kg Pb in environmental soil samples contaminated with Pb and other heavy metals, demonstrating its utility in quantifying bioavailable Pb (Van Der Lelie, Verschaeve, et al., 2000, Van Der Lelie, Tibarzawa, Corbisier, Vangronsveld, & Mench, 2000; Geebelen et al., 2003). In addition to Pb, *Cuprividus metallidurans* CH4 metal specific BIOMET® biosensor constructs were developed for Zn, Cd, Cr and Ni and Cu in *Cupriavidus silverii* DS185 (formally *Ralstonia silverii*) (Corbisier, Thiry, Masolijn, & Diels, 1994; Corbisier et al., 1999; Van Der Lelie, Verschaeve, et al., 2000; Van Der Lelie, Tibarzawa, et al., 2000). While specificity was not examined, a plasmid harboring the *pbrR* gene, it's promoter and green fluorescent protein (GFP) reporter gene was introduced into *Escherichia coli* WM3064, *Pseudomonas aeruginosa* PAO1, *Shewanella oneidensis* and two *Enterobacter* sp. and the whole-cell biosensors were able to detect Pb in tap and ground water (Bereza-Malcolm, Aracic, & Franks, 2016). When *pbrR* and *pbr*A were inserted into a *Pseudomonas fluorescens* plasmid or chromosome with the *lux* reporter, Hg, Cd, and Zn also were detected (Corbisier et al., 1999). Many of these microbial biosensors, such as *Shewanella oneidensis*, *Enterobacter* sp., *Pseudomonas aeruginosa*, may be better adapted for environmental use. While they use the same genetic elements, host strains and constructs are not identical, and this may account for differences in specificity.

Whole-cell Pb biosensors have been developed using other genes involved in heavy metal resistance however specificity is compromised. P_{1B}-type ATPase efflux pumps, such as the *pbr*A gene product in *Cupriavidus metallidurans*, can confer Pb, Zn, and Cd resistance (Lee, Glickmann, & Cooksey, 2001; Liu, Dutta, Stemmler, & Mitra, 2006; Rensing et al., 1998). The *Staphylococcus aureus* and *Pseudomonas putida* efflux pump is encoded by *cadA*; its homolog in *Escherichia coli* is *zntA* (Lee et al., 2001; Liu et al., 2006; Rensing et al., 1997; 1998). *Staphylococcus aureus cadA* conferred Pb resistance; however, results were mixed in *Pseudomonas putida* (Lee et al., 2001; Leedjarv, Ivask, & Virta, 2008). Several *Pseudomonas fluorescens* heavy metal biosensors that harbor *cadR* (receptor) and *cadA* (P-type ATPase) inducible by Pb have been developed. However, they also detected Hg, Cd, and Zn (Ivask, Rolova, & Kahru,

Table 7. Selected lead bioreporters and host strains in in vitro experiments and natural environmental samples.

Bacterial Host Strain	Sensor-Reporter Element	Pb^{2+} Level of Detection (Sensitivity) μM	Other Heavy Metals Detected (Specificity)	Reference
In vitro				
Alcaligenes eutrophus[a] AE2448	***pbrRPO*Δ*pbrA*::*lux***[d]	0.5	N.D.[c]	Corbisier et al., 1999
Pseudomonas fluorescens OS8	pDN***pbrRPpbrA****lux*	0.9	Hg, Cd, Zn	Ivask et al., 2009
Pseudomonas fluorescens OS8	Kn***pbrRPpbrA****lux*	0.2	Hg, Cd, Zn	Ivask et al., 2009
Pseudomonas fluorescens OS8	pDN***zntRPzntA****lux*	0.4	Hg, Cd, Zn	Ivask et al., 2009
Pseudomonas fluorescens OS8	pDN***cadRPcadA****lux*	0.4	Hg, Cd, Zn	Ivask et al., 2009
Staphyloccus aureus RN4220	pTO024[f]	0.033	Cd, Sb Antimony	Tauriainen et al., 1998
Pseudomonas putida KT2440	pDN***Pczc1****lux*	0.9	Zn, Cd	Hynninen et al., 2010
Pseudomonas putida KT2440.2431[g]	pDN***Pczc1****lux*	0.02	Zn, Cd	Hynninen et al., 2010
Escherichia coli MG1655	p***ZNT****lux*	0.03	Cd, Zn, Hg, Co, Ni, Sb, Cr	Riether et al., 2001
Escherichia coli	pSB1A2:: *LppOmpA****PbrRP***RFP[i]	0.001	Pb	Wei et al., 2014
Escherichia coli	pSB1A2:: *OmpA****PbrRP***RFP	0.001	Pb	Wei et al., 2014
Pseudomonas aeruginosa PAO1	pBB***pbrRgfp***[j]	0.2 μg/ml	Pb	Bereza-Malcolm et al., 2016
Enterobacter sp	pBB***pbrRgfp***	1.0 μg/ml	Pb	Bereza-Malcolm et al., 2016
Shewanella oneidensis	pBB***pbrRgfp***	0.5 μg/ml $Pb(NO_3)_2$	Pb	Bereza-Malcolm et al., 2016
Escherichia coli S23	pDGΔ*pbrAP*$_{pbrRT}$*pbrRgfp*	0.001	Pb	Jia et al., 2018
Escherichia coli DH5α	P558***pbrRP***$_{pbr}$*egfp* (pbrR558)	0.05	Pb	Du et al., 2019
Environmental samples				
BIOMET® Pb biosensor	***pbrRPpbrA****lux*	7 – 404 mg/kg Soil +/− Pb^{2+} amendment[h]		Geebelen et al., 2003
Cupriavidus metallidurans	***zntAP****lux*	1.2 – 4 ng/L Water	N.D.	Zhang et al., 2017
Escherichia coli	pSB1A2:: *LppOmpA****PbrRP***RFP[i]	0.001	Pb	Wei et al.,2014
Escherichia coli	pSB1A2:: *OmpA****PbrRP***RFP	0.001	Pb	Wei et al., 2014
Pseudomonas aeruginosa PAO1	pBB***pbrRgfp***[j]	1 & 5 μg/mL Tap or Ground water	Pb	Bereza-Malcolm et al., 2016
Enterobacter sp	pBB***pbrRgfp***	1 & 5 μg/mL Tap or Ground water	Pb	Bereza-Malcolm et al., 2016
Shewanella oneidensis	pBB***pbrRgfp***	1 μg/mL Tap or Ground water	Pb	Bereza-Malcolm et al., 2016
Escherichia coli DH5α	P558***pbrRP***$_{pbr}$*egfp* (pbrR558)	Sewage sludge 4.8 μM[l]	Pb	Du et al., 2019

[a]Cupriavidus metallidurans (Betaproteobacteria); formally Alcaligenes eutrophus, Wautersia metallidurans, Ralstonia metallidurans).
[b]Tn4431 inserted 1.4 kb downstream of czc region in cupS gene.
[c]N.D., not detected.
[d]Promoter/Operator region of pbrR. Also referenced as AE2450 or BIOMET® (Pb(II); Van der Lelie, Tibarzawa, et al., 2000).
[e]N.A., not available.
[f]cadA.
[g]Disrupted chromosomal P-type ATPase (cadA1, cadA2) and CBA transporter (czcCBA1, czcCBA2). Note that Pb2+ did not induce luciferase production in strains with pDNPcadA1lux.
[h]BIOMET assay determined Pb(II) concentration.
[i]Lpp, prolipoprotein; OmpA, outer-membrane protein A; RFP, red fluorescent protein; pbrR expressed on cell surface.
[j]Green fluorescent protein gene; gfp.
[k]Promoter 558, P558; Enhanced green fluorescent protein gene; egfp.
[l]No range of Pb concentration provided; authors state that Chinese standard for sewage effluent (4.8 μM) could be detected.

2009). *CadA Pseudmonas putida* biosensors were not inducible by Pb; yet they detected Zn (Hynninen et al., 2010). Chromosomal insertion slightly improved Pb detection performance. Similar detection levels were observed in *Staphylococcus aureus* RN4220 harboring a *cadAlux* constructed plasmid that also understand the detected Cd and antimony (Sb; Tauriainen, Karp, Chang, & Virta, 1998).

Another biosensor approach utilizes *zntA* and *zntR* (receptor), the Zn/Cd/Pb/Hg transporting ATPase, involved in heavy metal resistance (Ivask et al., 2009). This construct showed good sensitivity to Pb (*Pseudomonas fluorescens*) and also detected Hg, Cd, and Zn (Ivask et al., 2009). In *Escherichia coli* MG1655 harboring plasmid pZNTlux, Pb detection improved 10 fold; however, this biosensor also detected Cd, Zn, Hg, Co, Ni, Sb, and Cr (Riether, Dollard, & Billard, 2001). Zhang et al. (2017) developed a *zntAlux* biosensor that detected Pb in environmental water samples, however specificity was not tested.

The *czc*1 gene product, Co/Zn/Cd efflux permease (CBA transporter), when incorporated into a plasmid with *lux* operon in *Pseudmonas putida* KT2440, detected Pb, Zn, and Cd (Hynninen et al., 2010). Interestingly, in the *cadA1*, *cadA2*, *czcCBA1*, and *czcCBA2* deletion strain *Pseudomonas putida* KT2440.2431, Pb sensor sensitivity improved 45 fold (LOD 0.02 µM), possibly because chromosomal genes were not binding available Pb or, because *czc*CBA1 was involved in Pb export, higher levels of Pb remained in the cell (Hynninen et al., 2010; Leedjarv et al., 2008). *Cuprividus metallidurans* AE1433, which has a transposon (Tn4431, promoterless *lux* operon) inserted 1.4 kb downstream of the *czc* region in the *cup*S gene, detected Pb in incinerator fly-ash as well as Zn, Cd, and Co (Corbisier, Thiry, & Diels, 1996). If the goal is to detect multiple heavy metals, then engineering a biosensor that harbors a gene with shared functionality is a useful approach. However, if more specificity is required (i.e. detection of a specific metal such as Pb), then identifying a gene(s) unique to the metal may be preferred.

More recently, innovative approaches have been used to improve specificity and sensitivity of Pb whole-cell biosensors. Wei et al. (2014) developed a Pb specific biosensor that detected 0.001 µM Pb. It expressed the *pbrR* gene product and promoter on the cell surface by fusing prolipoprotein (l*pp*) and/or outer-membrane A (*ompA*) protein and the reporter, red fluorescence protein (RFP). Not only could the sensor detect Pb, other metals, such as Cd could be adsorbed providing a less toxic environment as demonstrated by *Arabidopsis thaliana* growth in heavy metal contaminated soil in its presence. Other researchers have manipulated promoter type and position. By placing *pbrR* and *gfp* under the *pbrRT* promotor (as opposed to the divergent *pbrABCD* promoter region) and incorporating positive

feedback loops, Jia, Zhao, Liu, Bu, and Wu (2018) were able to enhance the reporter signal and demonstrate 0.001 μM sensitivity. Incorporation of the moderately constitutive promoter P55 to control *pbrR* expression and placing *egfp* (enhanced green fluorescent protein gene) divergently under control of the *pbr* promoter (P_{pbr}) resulted in a dynamic detection range of 50 nM to 10 μM (Du et al., 2019). The sensor was able to detect 4.8 μM Pb, the Chinese sewage discharge standard, in highly complex sewage sludge. In another study, sensitivity optimization was demonstrated by tuning promoter expression strength. Testing different constitutive promoters for expression efficiency of *pbrR* maximized sensitivity when *efgp* was under the control of the PbrR protein binding promotor, P_{pbr} (Guo et al., 2019). These "gene circuit engineering" approaches, which manipulate genetic code to maximize outcome, in this case Pb detection, holds promise for future optimized specific sensors that will provide a more complete view of bioavailable environmental contamination, thus better informing ecological risk assessment.

Biologically based Pb and other heavy metal detection approaches continue to evolve with technology advances. In the 1980s and 1990s, colony hybridization with radiolabeled metal resistance gene probes was used to identify genes from related to heavy metal resistance such as *mer* (Hg resistance) and *czc* (Cd-Co-Zn resistance) from bacterial isolates and soils (Barkay, Fouts, & Olson, 1985; Diels & Mergeay, 1990). As technology advanced, PCR amplification of genes from environmental sources coupled with DNA hybridization illustrated presence of target genes in the environment (Trajanovska, Britz, & Bhave, 1997). With the advent of metagenomic sequencing and associated bioinformatics analysis, environmental resistance genes not only were identified in soils and sediments but also linked to microbial phyla (Collins-Fairclough, Co, Ellis, & Hug, 2018; Dunivin, Yeh, & Shade, 2019; Reddy & Dubey, 2019; Xavier et al., 2019). Pb and other heavy metal, antibiotic and biocide resistance genes were found in metagenomes from a river adjacent to a municipal waste leachate pond (Collins-Fairclough et al., 2018). The *pbrA* Pb resistance gene was identified in metagenomes from Ganges River (India) water and sediment along with genes that conferred resistance to Cu, Zn, Fe, Cr, As, Co, Ni, Cd, tellurium (Te), antimony (Sb), Hg and W (Reddy & Dubey, 2019). Soil from other sources yielded As (*arc3*, *aioA*, *arsB, arsC[grx], arsC [trx], arsD, arsM, arrA and arxA)*, Zn (*czc*D, *czc*B, *ZIP*, sigma-54 zinc dependent regulator were most abundant) and Cu (*copA*) resistance genes (Dunivin et al., 2019; Xavier et al., 2019). Quantitative PCR (qPCR) targeting specific resistance genes, such as *pbrR* which is utilized in construction of Pb whole-cell biosensors, may be a good approach to more rapidly identify an increase in gene number indicative of Pb contamination and suggestive of

bioavailability. With that said, metagenomics and qPCR approaches based on DNA do not differentiate genomes from live and dead cells; genome material from dead cells may be included in the analysis and skew results. Both approaches have considerable time and financial costs and may not be appropriate for rapid screening. Using a physiologically active whole-cell approach may be a faster, less expensive and better predictor of Pb and other contaminant bioavailability.

5. Conclusions and future perspectives

While it has long been recognized that exposure to Pb contamination is a critical stressor to microorganisms, the spatiotemporal variability of the microbial community and experimental uncertainties associated with the measurement of microbial communities in the laboratory were primary reasons for not considering microbial communities in screening level ecological risk assessments of Superfund sites (U.S. EPA, 2003). Information presented in this review provides reasonable evidence to reevaluate the status of the science and its application to risk assessment, as discussed below.

First, in an apparent response to varying levels of Pb stress, microorganisms can initiate a series of Pb resistance and adaptation mechanisms, even at a very early stage of exposure in the presence of low Pb concentrations. It is well known that microbial Pb resistance can be accomplished through intracellular and/or extracellular precipitation, adsorption, complexation and efflux pumps (Tables 1 and 2). Although identifying a unified set of Pb resistant microorganisms among different sites can be challenging due to variable site conditions, some Pb resistant bacteria such as the Gram negative *Proteobacteria* and Gram positive *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* were found to be common and dominant in many contaminated sites. These phyla also were found in piping biofilms where microorganisms are exposed to Pb contamination due to corrosion of Pb pipes (White, Tancos, & Lytle, 2011). Thus, an important future research area is to examine Pb resistant bacteria and their genes as benchmark indictors for evaluation of Pb contamination and toxicity, because microbial Pb toxicity is directly dependent upon Pb bioavailability. Varying levels of bioavailable Pb could trigger different resistance and adaptation strategies possessed by different bacterial strains in the community. In other words, the genes bacteria use to adapt to a specific level or range of environmentally bioavailable Pb offer a means to evaluate the ecotoxicological effects at contaminated sites. Thus, quantifying the relationship between the bioavailable portion of total Pb and microbial ecotoxicity and Pb resistance genes of benchmark bacteria can be very useful for site assessment. Carefully

designed *in vitro* studies with varying magnitude and duration of exposure can be conducted in the laboratory to help achieve this goal.

Second, the current literature, including both *in vitro* and *in situ* studies, indicated that exposure to Pb contamination inhibited microbial activity resulting in reduced respiration, suppressed metabolism, and reduced biomass as well as altered microbial community structure (Tables 3–5; de Vries et al., 2007; Xu et al., 2019). While most *in vitro* Pb incubation studies were not long enough to reach equilibrium and represent field conditions, findings from them were generally in agreement with conclusions from long term *in situ* studies of Pb and other heavy metals in terms of Pb toxicity and impacts on microbial community structure and activities (Table 3). Although other physical, chemical, and biological factors in the field such as temperature, moisture, other concomitant heavy metals, pH, particle size distribution, organic C content, and vegetation types could mask microbial community shifts associated with Pb contamination, the latest advancements in molecular techniques (e.g., 16S rRNA gene sequencing, functional gene microarray; metagenomic sequencing) coupled with statistical and network analyses can provide detailed phylogenetic analyses, functional gene identification, and discernment of the effects of Pb and other heavy metals on microbial communities (Tables 4–5; Jacquiod et al., 2018). Even at sites where microbial communities were compositionally similar, long-term heavy metal exposure resulted in functional differences regardless of the contamination level. Gene transfer and DNA recombination were more prevalent, thus providing a selective advantage at heavily contaminated sites more so than at less contaminated sites. While genomic approaches will continue to draw research interest in the scientific community, an emphasis of future research for consideration is standardizing the methodology for potential application in ecological risk assessments of contaminated sites. To that end, a unified molecular methodology for analysis of microbial community structure and functional properties should be developed and tested with samples representing varying spatiotemporal scales coupled with the determination of total and bioavailable Pb. Interlaboratory comparison of the resulting methodology can determine experimental variability and help explain measurement uncertainties, leading to a reduction in uncertainty. The resulting microbial community shifts can serve as indices of Pb contamination and bioavailability and inform both human health and ecological risk assessments.

Lastly, the latest molecular understanding of Pb resistance mechanisms has identified suites of functional genes responsible for specific Pb resistance mechanisms. These resistance genes may serve as indicators of Pb contamination in association with dominance of certain microbial phyla. This advanced understanding has led to the development of several Pb

biosensors in the realm of environmental biotechnology (Table 7). Using biosensors containing Pb resistance genes may provide an indication of Pb bioavailability because the microbes can sense biologically available Pb (as opposed to unavailable, bound Pb). In deciding which sensor to use, specificity is a key consideration because some sensors can detect several metals while a few are specific for Pb. Nevertheless, the biosensor approach can be less costly to use as a screening assay and has application in Pb bioavailability for ecological risk assessment. Future research should be directed to validate the effectiveness and reliability of these sensors with conventional methods for bioavailable Pb detection.

In conclusion, microorganisms play an important role in Pb biogeochemistry and have adapted to populate niches left available by Pb sensitive microbes following contamination events. With a better understanding of the Pb induced community shift dynamics, including prevalent resistance mechanisms, better insight into Pb toxicological effects on both community structure and function can be ascertained. Exploiting these organisms at the molecular, population, and community levels has the potential to better predict bioavailable Pb levels, thereby strengthening both human and ecological risk assessments.

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