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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 waves of studies on responses of microorganisms to lead contamination exposure, leveraging detailed phylogenetic analyses and functional gene identification to discern the effects of lead toxicity on microbial communities. This work provides a comprehensive review of recent research on (1) microbial community changes in contaminated aquatic sediments and terrestrial soils; (2) lead resistance mechanisms; and (3) using lead resistance genes for lead biosensor development. Sufficient evidence in the literature, including both *in vitro* and *in situ* studies, indicates that exposure to lead contamination inhibits microbial activity resulting in reduced respiration, suppressed metabolism, and reduced biomass as well as altered microbial community structure. Even at sites where microbial communities do not vary compositionally with contamination levels due to extremely long periods of exposure, functional differences between microbial communities are evident, indicating that some microorganisms are susceptible to lead toxicity as others develop resistance mechanisms to survive in lead contaminated environments. The main mechanisms of lead resistance involve extracellular and intracellular biosorption, precipitation, complexation, and/or efflux pumps. These lead resistance mechanisms are associated with suites of genes responsible for specific lead resistance mechanisms and may serving as indicators of lead contamination in association with dominance of certain phyla. This allows for development of several lead biosensors in environmental biotechnology. To promote applications of these advanced understandings, molecular techniques, and lead biosensor technology, perspectives of future work on using microbial indicators for site ecological assessment is presented.

Graphical Abstract

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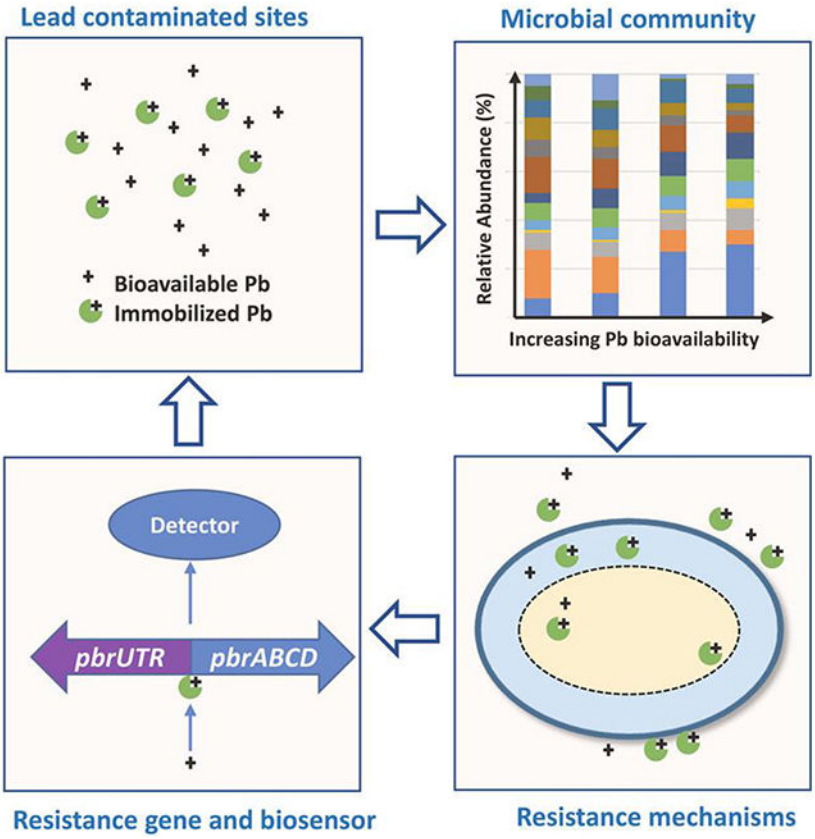

Keywords

Lead contamination; microbial community; lead resistance; lead biosensors; ecological risk assessment

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 et al., 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 lead levels have significantly increased (U.S. DHHS/ATSDR, 2007). Lead is mined worldwide and purified through smelting, which releases lead into the environment in the form of hazardous fumes, fallout, and dust (WHO/IARC, 2006; U.S. DHHS, 2007). Total lead concentrations on contaminated sites can reach up to 10 000 mg/kg, while the average value in natural soils ranges from 10 to 50 mg/kg. Pb can be found as a co-contaminant with other heavy metals such as aresenic (As), cadmium (Cd), cobalt (Co), copper (Cu), nickel (Ni) and zinc (Zn). Aside from mining and smelting activities, sequestered lead 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 USA, it was used as a paint pigment until 1978, and as lead solder for food cans and an "anti-knock" agent in gasoline until 1995 (U.S. DHHS/ATSDR, 2007). Industrial waste can be contaminated with lead and other heavy metals, which in turn, pollute the surrounding environment (Jiang et al., 2019; Kuppusamy et al., 2016; Li et al., 2017; Shi et al., 2002). Banning lead as a fuel additive resulted in decreasing air concentrations. However, because lead does not degrade in the environment, lead based paint and contaminated soils 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 (U.S. DHHS, 2007; WHO, 2011; WHO/IARC, 2006). Many countries regulate environmental lead (Tong et al., 2000). In the United States, the Environmental Protection Agency (U.S. EPA) stipulates that the National Ambient Air Quality Standard for lead is 0.15 μg/m^3 total suspended particles (Fed Register, 2008; Fed Register, 2016) and 15 μg/L in drinking water (U.S. EPA, 2008). The US EPA's standard for lead in bare soil in play areas is 400 mg/kg and 1200 mg/kg for non-play areas (U.S. EPA, 2001). Lead is frequently found as a co-contaminant at Superfund sites; there is no reference dose (RfD) for lead, and the risk reduction goal used for cleanup is based on the probability that ≤ 5% children will have a blood lead concentration of ≥ 10 μg/dL (U.S. EPA, 2019).

Cleanup of lead contamination at U.S. Superfund sites includes removal of material, groundwater treatment, engineering controls and other approaches focused on reducing the risk of lead exposure, especially to children, consistent with the U.S. Federal Lead Action Plan (U.S. Executive Office of the President, 2018). As part of the risk assessment, lead bioaccessibility, bioavailability, bioaccumulation, and bioconcentration are considered (U.S. EPA, 2007). Currently, the ecological risk assessment considers higher level organism effects and remains silent on microorganisms and microbial processes due to considerable spatial and temporal variation and experimental uncertainties (U.S. EPA, 2003). However, because microorganisms play a critical role in carbon and nitrogen cycling and other fundamental geochemical processes, groups of European scientists advocate for ecological risk assessments protective of microorganisms (Dahlin, Witter, Martensson et al., 1997; de Vries et al., 2007; Giller, Witter, & McGrath, 2009). Therefore, it is important to understand the impact of lead contamination on microbial community.

Lead is not biologically essential and can be toxic to microorganisms at low concentrations. Once exposed to lead contamination, microorganisms develop a variety of resistance mechanisms that are selective in high lead concentration areas, resulting in an increase in lead resistance genes and changes in microbial community activity and composition (Braud et al., 2009; Naik & Dubey, 2011; O'Brien et al., 2014). These resistance genes have been harnessed in lead biosensors to detect bioavailable lead (Hobman, Julian, & Brown, 2012; Zhang et al.,2017). Furthermore, microbial processes can form insoluble lead precipitates, thus reducing lead bioaccessibility and bioavailability. Twenty-five years ago, microbial process studies focused on measuring respiration, biomass, litter decomposition and enzyme synthesis and activity, and using those end points, critical toxicity concentrations of lead and other heavy metals could be determined (de Vries et al., 2007). Microbial community

diversity studies, comparing contaminated and uncontaminated soils and water, used microscopy, plate counting and phospholipid fatty acid analysis (Dahlin et al., 1997; Fakruddin & Mannan, 2013). As molecular approaches, such as 16S rRNA analysis, became more commonplace, higher resolution in microbial community composition was possible. While uncertainties including temporal and spatial variation remain, quantifying microbial resistance genes, indicative of increased environmental lead load or changes in microbial diversity using molecular approaches, can serve as indices of lead contamination and bioavailability to inform both human health and ecological risk assessments.

The objective of this work is to provide a critical review of recent research on the impact of lead contamination on microbial community structure and function as well as lead resistance mechanisms and their use for lead biosensor development for potential application in bioavailability assessment of contaminated sites. The specific objectives are as follows: 1) to evaluate the impact of lead contamination on microbial community structure and function in contaminated terrestrial soils and aquatic sediments; 2) to elucidate various lead resistance mechanisms; and 3) to leverage resistance genes for lead biosensor development to detect lead and assess lead bioavailability. Perspectives of future work on using microbial indicators for site ecological risk assessment is presented.

2. Impact of lead contamination on microbial community structure and function

Understanding the effect of lead contamination on microbial communities, including activity and community composition, is important because microorganisms may serve as a direct or indirect indicator of ecosystem changes or effect of perturbations. Microbial lead 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). Thus, suppressed microbial activity is typically observed. For example, in lotic sediments contaminated with heavy metals (Cd, Cr, Cu, Ni, Pb, Zn) from mixed sources (e.g. treated and untreated industrial effluents, urban sewage, atmospheric deposition, rain waste waters), microbial respiration and biomass are negatively correlated with heavy metal concentration (Jaiswal & Pandey, 2018). Microbial activity is assayed by measuring fluorescein diacetate hydrolytic activity, a surrogate for lipase, protease, esterase, alkaline phosphatase, and β-D-glucosidase. Except for alkaline phosphatase (due to high concentrations of phosphate), the other parameters are negatively correlated with heavy metal concentration (Green et al., 2006; Jaiswal & Pandey, 2018). Both bioavailability and toxicity of metals are also negatively correlated with organic matter, possibly reflecting complexation of heavy metals by organic materials (ref).

While lead is bacteriostatic or bactericidal to many microorganisms, some have developed resistance mechanisms that enable them to survive or thrive. Comparative studies between varying levels of contamination often reveal changes in microbial community structure and function (e.g., Akmal et al., 2005; Liu, Lin, Dong, Li, & Liu, 2018). Conventionally, alpha and beta diversity, respiration, biomass, or metabolic enzymes are measured to infer microbial community changes. For example, measurement of phospholipid fatty acids

(PLFA) has been used to identify microbial community structure alteration due to metal toxicity (Lenart & Wolny-Koladka, 2013; Zhang et al., 2018). However, PLFA analysis does not provide detailed quantitative information about microbial community structure, albeit insights from a few biomarker fatty acids indicative of a broad change can be gained. 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) have allowed for detailed phylogenetic analyses, functional gene identification, and discernment of the effects of lead and other heavy metals on microbial communities (Chen et al., 2018). For example, Functional Response Group (FRG) analysis, which is based on RNA and DNA abundance patterns instead of phylogeny, can functionally characterize microbial communities in terms of their metal tolerant nature in a contaminated environment (Jacquiod et al., 2018).

This section is based on a review of available literature relating lead and other heavy metal contamination with microbial community structure and function in aquatic sediments and terrestrial soils (Tables 1–3). While several of the evaluated studies are conducted through short-term *in vitro* experiments whereby lead exposure is artificially imposed by incubation with lead nitrate in a green house or laboratory setting (Table 1), most studies involve *in situ* field sampling of contaminated sites that have a contamination history of decades to over one hundred years (Tables 2 & 3). In these contaminated sites, lead typically co-exists with other heavy metals. Thus, most *in situ* studies on microbial communities reflect a collective effect of a group of heavy metals. Because the concentrations of different heavy metals in these contaminated sites often covary, determination of the effects of any individual heavy metal contaminant on microbial community can be challenging. In spite of limited *in situ* studies on lead-specific ecological and toxicological effects, the impact of lead exposure can be inferred because lead is one of the major heavy metal contaminants and many of them share the same toxicological effects on microbial community (Xu et al., 2019). The *In vitro* studies that used lead dosing and incubation help to better isolate lead-specific ecological and toxicological effects.

2.1 Microbial community under early stage exposure to lead contamination

Several studies have indicated that early stage exposure to lead contamination results in suppressed microbial metabolism, reduced biomass production in association with higher energy demand, and changes in diversity and relative abundance (Table 1; Akmal et al., 2005; Liu et al., 2018; Sobolev & Begonia, 2008; Xu et al., 2018). The immediate inhibitory effects are typically reflected in carbon 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 due to a heavy metal spill (Cd, Zn, Pb, Cu, As; China) showed reduced abundance of the nitrous oxide reductase gene, *nosZ*, suggesting lower denitrifier community richness (Table 2; Guo et al., 2018). Following the spill, the dominant *nosZ*-denitrifier genus observed is *Pseudogulbenkiania* (*Betaproteobacteria*), whereas the genus *Pseudomonas* (*Gammaproteobacteria*) increases following the spill and two other unidentified denitrifier groups decrease (Guo et al., 2018), suggesting that the some members of the microbial community react to contamination of lead and other heavy metals, in part, by developing metal-resistant mechanisms while others are susceptible. The

selection of microorganisms for lead-resistant forms of nitrite reductase in early stage exposure to lead contamination is also evidenced by changes in the community harboring *nir*K in lead nitrate amended soil incubated for 18 months at 18-24°C (Sobolev & Begonia, 2008). This study further notes that lead has detectable effects upon the community diversity at relatively low concentrations, and there are several thresholds as concentrations increase, each causing a shift in microbial diversity. Several other studies have also reported microbial community shifts in lead amended soil (Liu et al., 2018; Xu et al., 2018). In another study with lead nitrate amended soil, incubated for 56 days at 25°C, utilization of L-phenylalanine, D-mannitol, α-ketobutyric acid increased while Tween40, pyruvic acid methyl-ester, hydroxy butyric acid, itaconic acid utilization decreased, suggesting alteration in microbial community-level metabolism and activity (Akmal et al., 2005).

In the presence of lead, resistant microorganisms clearly have a selective advantage due to their ability to persist in a lead contaminated environment which selects against susceptible microbes. Soil incubated with 5000 mg/kg lead nitrate for 49 days results 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 is unaffected based on their increased numbers in the lead spiked soil. Upon the addition of macadamia nutshell biochar, which reduces lead bioavailability, fungi and actinomycetes recover slightly and microbial respiration and biomass production increases (Xu et al., 2018). When lead amended soil is acclimated for 4 weeks, the dominant phyla observed are *Bacteroidetes, Proteobacteria, Firmicutes, and Actinobacteria*. *Acidobacteria, Verrucomicrobia, and Chloroflexi* are less abundant. However, as the lead concentration decreases during phytoremediation, these latter phyla increased in abundance. At the genus level, *Bacillus, Adhaeribacter, Pontibacter, Flavisolibacter*, and *Kaistobacter* are present in the lead amended soil. Following phytoremediation, *Flavisolibacter, Kaistobacter,* and *Pseudomonas* increase in abundance with a relative decrease in *Bacillus, Adhaeribacter, Pontibacter*, and *Paenibacillus*, thus suggesting reduced lead tolerance. Bioavailability and bioremediation clearly influence the community structure in lead amended soil (Liu et al., 2018; Xu et al., 2018).

2.2 Microbial community under long-term exposure to lead contamination: aquatic sediments

Sediment microbial communities in aquatic systems adjacent to mining and smelting operations are impacted by heavy metal contamination, with lead identified as a primary constituent (Table 2; Feris et al., 2003; Gillan, Danis, Pernet, Joly, & Dubois, 2005; Jie et al., 2016; Roosa, Wattiez, et al., 2014; Zhang, Xu, Zhao, Rong, & Zhang, 2018). In sediments contaminated from copper (USA) and tungsten (China) mining operations which contain significant quantities of lead, dissimilarity in microbial communities along a chemical concentration gradient increases as metal content increased (Feris et al., 2003; Zhang et al., 2018). However, in contaminated sediments due to copper mining activity, biomass is unaffected by metal concentration with no apparent correlation between sediment metal content and diversity or total productivity (Feris et al., 2003), suggesting that other factors controlling biomass outweigh the impact of heavy metal contamination. Rather, the structure of microbial communities is significantly affected. Phospholipid fatty acid analysis indicates

that heavy metal concentration positively correlates with prokaryote abundance and eukaryotes and actinomycetes negatively correlate. Further analysis by qPCR using specific primers reveals a positive correlation with *Gammaproteobacteria* and a negative correlation with *Betaproteobacteria* abundance (Feris et al., 2003). In the tungsten mining site (China), 16S rRNA gene sequencing identifies *Proteobacteria* and *Actinobacteria* as the dominant sediment phyla (Zhang et al., 2018). While all sites with varying concentrations of heavy metals have unique members, they also share many members in common. Zhang et al. (2018) confirms the negative correlation of metal concentration with *Betaproteobacteria* and *Deltaproteobacteria* while members of the phyla *Acidobacteria*, *Latescibacteria*, *Verrucomicrobia*, *Nitrospirae* and *Gemmatimonadetes* are 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 2). Inductively coupled plasma atomic emission spectroscopy reveals that the sediments are contaminated with Cu, Pb, Zn, As, Cd, Ni, Hg, Cr, Mn, Co, and S, 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 have lower Shannon diversity and Pielou eveness indices are dissimilar to other less contaminated sediments (Ren et al., 2016; Yin et al., 2015). Members of the phyla *Firmicutes*, *Chloroflexi* and *Crenarchaeota* are more abundant in the highly contaminated sediments whereas *Proteobacteria* and *Actinobacteria* are present but in lower abundance (Yin et al., 2015). Molecular ecological network analysis reveals that Pb, as well as mercury (Hg), Zn and carbon (C), correlate with the network module containing the phyla *Bacteroidetes, Chloroflexi, Proteobacteria, Acidobacteria, Firmicutes* and *Actinobacteria* from the highly contaminated sediments, thus suggesting microbial community composition and co-occurrence of phyla, is driven by the heavy metals in the sediment (Yin et al., 2015). Dominant microbial genera in all sediments along the gradient are *Fusibacter*, *Janthinobacterium*, *Proteiniclasticum*, *Acinetobacter*, and *Massilia* with ~15-18% unclassified to the genus level. *Fusibacter*, *Geobacter*, and *Proteiniclasticum* are more abundant in sediments with higher heavy metal concentration whereas *Janthinobacterium*, *Arthrobacter*, *Sphingomonas* and *Flavobacterium* are at higher numbers in the 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 lead resistant genes abundance correlated with lead concentration across the gradient (Jie et al., 2016). In the most heavily contaminated sediment, genes encoding for heavy metal resistance and carbon cycling pathways, to include 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 2; Li et al., 2006; Chen et al., 2018). Lead concentrations are in the same range (153 ppm vs 124 ppm) as those observed in the Xiangjian River site, with other heavy metals present at

both sites at comparable levels (Chen et al., 2018; Ren et al., 2016). Microbial community diversity is negatively correlated with heavy metal concentration and carries a higher viral load than sediments from less contaminated sites. The phyla *Proteobacteria*, *Cyanobacteria*, *Tenericutes*, *Firmicutes*, and *Bacteroidetes* abundance show a positive correlation with heavy metal contamination while *Verrucomicrobia*, *Actinobacteria* and *Chloroflexi* are less represented (Chen et al., 2018). *Proteobacteria, Bacteroidetes*, *Firmicutes* are the most dominant phyla harboring heavy metal resistance and reduction genes However, heavy metal resistance and reduction genes are detected in the Bacteria *Gemmatimonadetes*, *Planctomycetes, Acidobacteria, Tenericutes, Spirochaetes, Chlorobi* and *Parubacteria* and the Archea *Euryarchaeota* and *Thaumarchaeota* using quantitative polymerase chain reaction (qPCR) analysis. Within the phylum *Proteobacteria*, the classes *Betaproteobacteria*, *Gammaproteobacteria*, and *Alphaproteobacteria* harbor the highest number of heavy metal resistance genes, and they are 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 are more prevalent in the more highly contaminated sediments.

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 2; Roosa et al., 2014; Roosa, Wauven, et al., 2014). Directly adjacent to the smelter, sediment lead concentration averages 913 mg/kg and co-occurs with aluminum (Al), As, Cd, Co, Cr, Cu, iron (Fe), manganese (Mn), Ni, Pb, vanadium (V), and Zn (Gillan et al., 2015). Lead concentrations are higher than in the most contaminated sediments of the Xiangjian River (737 vs 913 mg/kg; Jie et al., 2016). The upstream lead concentrations average 112 mg/kg, comparable to those observed in two of the Xiangjian River studies (Chen et al., 2018; Ren et al., 2016). Sediments from this site, and lessor contaminated sites upstream, have been studied extensively to elucidate the impact of heavy metal contamination on microbial community structure and function and better understand how these communities adapt to the presence of the heavy metals (Roosa, Wauven et al., 2014; Gillan et al., 2015; Jacquiod et al., 2018). At the phylum level, microbial communities in sediments adjacent to the smelter are similar to those in the upstream less contaminated site, possibly reflecting the effect of long-term exposure to contamination. This finding is similar to one from a study of marine sediment associated with 80 years of Zn and Pb smelter operation (Norway), which reports that microbial communities from contaminated sites are 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. While the metal contaminated site and upstream control sediments contain taxonomically similar microbial communities (70% similar), functionally they are different (Gillan et al., 2015). The higher-Pb sediments contain 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 are more prevalent in the higher-Pb sediments (Gillan et al., 2015). The change in the community genetic potential clearly shows a lead resistance selective advantage in the contaminated sediment. Betapr*oteobacteria* dominate at both lead contaminated sites, primarily by the genera

Burkholderia, Rubrivivax, Leptothrix, and Cupriavidus. Gammaproteobacteria (*Pseudomonas*) and *Alphaproteobacteria* (*Methylobacterium*) also are represented. *Pseudomonas, Thiobacillus, Acidovorax, Dechloromonas, Pseudoxanthomonas, Stenotrophomonas and Alicycliphilus* are more prevalent in the higher contaminated sediment whereas *Rubrivivax, Anaeromyxobacter, Leptothrix, Sorangium, Mycobacterium and Streptomyces* are more numerous in the less contaminated, upstream site (Gillan et al., 2015; Shi et al., 2002).

Roosa, Wauven et al. (2014) further noted that metal contaminated sediments contain microorgainsms from the phyla *Actinobacteria (Mycobacterium vaccaevaccae, Mycobacterium llatzerense, Rhodococcus erythreus, Streptomyces coelicoflavus), Alphaproteobactera (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)* which are lead and multiple heavy metal resistant even though they were originally selected on media supplemented with Pb, Cu, Ni, Cd, Co or Zn. The *Pseudomonas* community is positively correlated with concentrations of 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* q. The *pbr*T gene is present in the high-Pb sediments; however, correlation with Pb concentration is inconclusive (Roosa, Wattiez, et al., 2014). Interestingly, copy numbers of the *czc*A gene (encodes Co/Zn/Cd efflux pump) show a positive correlation with Pb concentration even though it is 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) confirms that the microbial community is dominated by *Proteobacteria* with *Firmicutes* the second most prevalent. *Alphaproteobacteria* and *Betaproteobacteria* are more numerous in metal contaminated sediments whereas *Gammaproteobacteria* are more abundant in control sediments. *Alphaproteobacteria* 16S rRNA levels (cDNA) relative to 16S rRNA gene (DNA) levels (RNA/DNA) are higher in metal contaminated sediment, implying increased activity; *Actinobacteria* have a lower OTU RNA/DNA ratio, suggesting sensitivity. The *Bacteroidetes* represent the passive part of community. *Ignavibacteriae*, *Deltaproterobacteria*, *Gemmatinmonadetes* and *Verrucomicrobia* are present in low abundance in the metal contaminated sediments. However, the three latter groups, as well as *Acidobacteria*, *Alphaproteobacteria*, *Betaproteobacteria*, *Nitrospirae*, *Planctomycetes*, have increased RNA/DNA ratios (Jacquiod et al., 2018). The Functional Response Group (FRG) analysis employed in this study reveals that the metal contaminated sediments contain “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.

2.2 Microbial community under long-term exposure to lead contamination: terrestrial soils

Lead and zinc mining and lead-containing industrial activities have occurred globally for decades and left an environmental footprint as contaminated soils that influence microbial biomass, activity, diversity, and community structure (Table 3; Azarbad et al., 2013; Beattie et al., 2018; Guo, Kang, & Feng, 2017; Hu, Qi, Zeng, & Zhang, 2007; Xu et al., 2017). For example, twenty years of heavy metal contamination explains the dissimilarity of microbial communities and bacterial abundance between contaminated and uncontaminated control soils (China; Hu et al., 2007). Bacterial abundance is negatively correlated with Pb, Cd, Zn, Mg whereas Archaea are positively correlated with pH, and Al, Pb, Cd, Zn significantly impact community composition (Beattie et al., 2018). Gram-positive bacteria positively correlate to heavy metal contamination and Gram-negative bacteria and fungi show a negative correlation (Azarbad et al., 2013). Organic matter has a stronger correlation than heavy metal content, suggesting a link with heavy metal bioavailability (Azarbad et al., 2013). However, at a site with over 100 years of mining operations and 40 years of lead and zinc enrichment, no apparent heavy metal induced differences in diversity or richness are observed (China; Xu et al., 2017). Biomass and respiration are negatively correlated with levels of nine metals including lead, accounting for differences in microbial community structure (Poland, USA; Azarbad et al., 2013; Beattie et al., 2018; Xu et al., 2017). At a lead battery recycling facility that contaminated soil for 40 years (lead 10,000 mg/kg; 5% water), microbial biomass and respiration are decreased. However, addition of a carbon source stimulates biomass production (Shi et al., 2002). Near a metallurgy plant (in operation since the 1970s), Pb, Cd, Cu and Zn contaminated soil also reduces microbial biomass and respiration as well as dehydrogenase activity (Chen et al., 2014).

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 soil contaminated for twenty years by industrial waste, community structure is statistically linked to heavy metal concentration unlike diversity richness and evenness are not (Xiaoqi Li et al., 2017). Archea abundance is positively correlated to Cd, As, Zn and Pb, and bacteria are negatively correlated to these metals (Li et al., 2017). *Acidobacteria*, *Ascomycota* and *Chytridiomycota* are inhibited in Cd, As, Zn and Pb contaminated soils (Chen et al., 2014). *Proteobacter, Crenarchaeota* and *Euryarchaeota* are more abundant in the heavy metal contaminated soil whereas *Chloroflexi* are more abundant in control soil (Li et al., 2017). Electronic waste and copper enriching 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* dominate (in descending order; Jiang et al., 2019). Physical and other chemical properties, such as organic matter levels and pH, influence microbial diversity (Jiang et al., 2019). Manufactured gas plants (Australia), in operation for 150-200 years, resulted in polycyclic aromatic hydrocarbon and heavy metal (Al, Cr, Ni, Cu, Zn, As, Cd, Pb) soil contamination which selects for *Proteobacteria* and other Gram-negative bacteria,

Gemmatimonadetes and *Bacteriodetes*, and influenced *Chloroflexi* which are present in lower abundance (Kuppusamy et al., 2016). At the site of a large mining operation which ceased in mid-1950s (USA), Al, Cd, Pb, and/or Zn show significant influence on selecting for 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) are associated heavy metal contamination in soils polluted by mining wastes in China (J. Guo et al., 2017). In another study, *Actinobacteria* and *Chloroflexi* are more dominant in the mine soil compared to control where *Verrucomicrobia* are 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 while sensitive ones are reduced.

The above studies also indicate that most bacteria living in extremely polluted soils belong to the class *Proteobacteria*. Guo et al., 2017 showed that at the genus level, *Sphinogomonas* is 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* correlate with Cd and Pb levels ($p<0.05$). *Ralstonia* and *Gemmatimonas* negatively correlate with Cd, Pb, As, Hg and pH; *Mizugakiibacter* and *Rhodanobacter* negatively correlate with Cd, Pb, As and soluble organic matter; and *Blastocatella*, an unidentified *Nitrospiraceae*, and an unidentified *Acidobacteria* positively correlate with Pb, Cd, Zn, Hg and soluble organic matter (Jing Guo, Yong Kang, & Ying Feng, 2017). In another lead and zinc enrichment site with many years of mining operation in China, Xu et al. (2017) indicate that below the phylum level, *Norcardioides*, *Gaiella*, *Comamonadaceae*, *Acidimicrobiaceae*, *Actinobacteria* and *Skermanella* are negatively correlated with metal concentrations and present in lesser numbers than in control samples. In contrast, abundances of *Verrucomicrobia* and *Bradyrhizobium* show positive correlations with Pb and Zn levels.

Approximately fifty years of nonferrous smelter operation, which ceased in 1989 (South Korea), resulted in enduring soil contamination by Pb and other heavy metals (As, Cd, Cu, Ni, Pb and Zn; Tipayno et al., 2018). In these soils, lead significantly correlates to the soil bacterial community composition at the phylum level, with relative abundance linked to pH. At the genus level, lead positively correlates with *Desulfatibacillum* and *Desulfovirga*; and negatively correlates with *Desulfococcus* (Tipayno et al., 2018). Analysis of community functional profiles (“Pathway abundance profiles”) reveals an increase in genes encoding enzymes associated with DNA replication and repair, translation, transcription, and nucleotide metabolism pathways in metal contaminated soil, whereas genes encoding enzymes associated with amino acid, lipid, and energy metabolism and biodegradation potential of xenobiotics are less abundant (Tipayno et al., 2018). As, Cd and Pb levels are positively correlated with enzymes associated with cell growth/death, transcription, signaling molecules, and interaction pathways; enzymes associated with transport, catabolism and metabolism of terpenoids and polyketides are negatively correlated.

3. Lead resistance mechanisms

Microorganisms have developed extracellular and intracellular strategies to persist in lead contaminated environments (reviewed by Jaroslawiecka & Piotrowska-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, Hodgson, & Buckling, 2014), ultimately resulting in lead precipitates, such as lead phosphates through phosphatase action. Surface biosorption (lipopolysaccharide, Gram negative; peptidoglycan, Gram positive) also excludes lead from the cell (Chang, Law, & Chang, 1997; Karimpour et al., 2018). Lead can enter the cell and either bind with metallothionein (Murthy, 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 lead precipitation (Borremans, Hobman,& Provoost et al., 2001; Hynninen, Touze, & Pitkanen, et al., 2009; Murthy, 2011; Naik, Shamim, et al., 2012c; Rensing, et al., 1998). These lead resistance mechanisms are schematically shown in Figure 1. Some of the extracellular and intracellular strategies are described in detail below.

3.1 Extracellular immobilization

Selected microbial species with extracellular biosorption functions reported in the literature are listed in Table 4. When exposed to lead contamination, microorganisms will firstly invoke extracellular immobilization strategies to limit the entry of lead ion [Pb(II)] into the cell envelop to maintain metal homeostasis. In general, extracellular immobilization is accomplished mainly through biosorption and precipitation. While extracellular precipitation of lead resembles intracellular precipitation, which is described in the next section, biosorptive binding of lead involves a series of polymers or compounds produced by microorganisms including exopolysaccharides, siderophores, and various functional groups on the cell wall (Figure 1).

Exopolysaccharides (EPS) are high molecular weight polyanionic polymers secreted by microorganisms into their environment. These extracellular polysaccharides protect the cell from lead 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, 1978; Roane, 1999). Transmission electron microscopy reveals lead accumulation in *Pseudomonas marginalis*, resisting 2.5 mM lead nitrate (Roane, 1999), and *Enterobacter cloacae* strain P2B, resisting 1.6 mM lead nitrate (Naik, Pandey et al., 2012a), is through binding to carboxyl, hydroxyl, and amide functional groups and glucuronic acid in EPS polymer chains. Glucuronic acid EPS is produced constitutively, such as in *Pseudomonas marginalis* (Roane, 1999) or induced in the presence of metals, including lead, in *Pseudomonas aeruginosa*, *Bacillus amyloliquefaciens*, *Bacillus subtilis* subsp. *subtilis*, *Enterobacter cloacae* strain P2B and other *Enterobacter* spp. (Bhaskar & Bhosle, 2006; Chowdhury et al., 2008; El-Shanshoury et al., 2012; Naik, Pandey et al., 2012a). A *Bacillus anthracis* isolated from industrial waste water excretes EPS and precipitates lead sulfide (PbS) extracellularly (El-Shanshoury et al., 2012). Purified EPS

from *Marinobacter* sp. unexposed to lead, binds lead and copper *in vitro* (Bhaskar & Bhosle, 2006). Immobilized biofilms, which contain polysaccharides and other biopolymers (reviewed by Marvasi et al., 2010), accumulate lead, 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 bioabsorb lead, possibly due to interactions with EPS (Chang et al., 1997; Karimpour et al., 2018).

Siderophores (Greek: “iron carrier”) 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 also can bind heavy metals, such as lead, and reduce toxicity (O’Brien et al., 2014). In *Pseudomonas aeruginosa* PAO1, lead binds to the siderophore pyoverdine but is not transported into the cell (Braud et al., 2009). Some heavy metals, such as Cd, Co, gallium (Ga), Hg, Mn and Zn, can inhibit iron uptake, however lead does not (Braud et al., 2009). Furthermore, it does not inhibit pyoverdine production (Braud et al., 2009). A lead resistant strain of *Pseudomonas aeruginosa* (strain 4EA), isolated from soil contaminated with lead battery waste, increases pyochelin and pyoverdine production coupled with reduced cell size in the presence of lead (Naik & Dubey, 2011). Scanning electron microscopy and energy dispersive X-ray spectroscopy reveal biosorption of lead (Naik & Dubey, 2011). Excreted siderophores also scavenge lead, resulting in the production of lead precipitates through phosphatase action and formation of lead 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, can 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 reveal biosorption of lead onto cell surface of *Pseudomonas aeruginosa*, isolated from soil contaminated with lead battery waste (Naik & Dubey, 2011). Therefore, these lead resistance bacterial strains may potentially serve as biosorbents for lead remediation.

3.2 Intracellular accumulation

When Pb(II) enters the cell under exposure to high concentrations, intracellular resistance strategies are triggered, first through an efflux pump, or metal transporting ATPases, to transport lead outside the cell membrane to the periplasm where lead can form insoluble precipitates lead through oxidation or reduction, thereby sequestering the lead and protecting the cell from its toxic effects. Meanwhile, metallothioneins can also bind lead 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 5.

Precipitates identified in lead-exposed microbes include lead(II) oxide (PbO; El-Shanshoury et al., 2012), lead(II) sulfide (PbS; De et al., 2008; El-Shanshoury et al., 2012; Essa, Al Abboud, & Khatib, 2018; Gong, Zhang, Bai, & Yang, 2007; X. Li et al., 2016), lead(II) sulfite ($PbSO_3$; Sharma et al., 2017), lead(II)sulfate ($PbSO_4$; X. Li et al., 2016), lead(II) carbonate ($PbCO_3$; Essa et al., 2018; Kang et al., 2015), lead(II) phosphate ($Pb(PO4)_2$; Borremans et al., 2001; Hynninen et al., 2009), and an unusual lead phosphate salt, $Pb_9(PO4)_6$, precipitated by *Vibrio harveyi* (Mire et al., 2004). Lead precipitates are typically formed in the periplasm, a concentrated gel-like matrix in the space between the inner cytoplasmic membrane and the bacterial outer membrane. Intracellular lead oxide (PbO) precipitation has been reported to occur in the periplasm of an *Enterobacter sp*. (El-Shanshoury et al., 2012). Sharma et al. (2017) reports periplasmic lead sulfite accumulation in *Providencia vermicola* strain SJ2A, isolated from a battery manufacturing plant. Lead sulfide (PbS) has been associated with lead 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 accumulates in the periplasm of *Desulfotomaculum* sp. (Gong et al., 2007) and *Bacillus megaterium*, isolated from a silver mining region (Roane, 1999). Accumulation of lead phosphate in the periplasm, by *Cupriavidus metallidurans* strain CH34 (formerly *Alcaligenes eutrophus, Wautersia metallidurans,* and *Ralstonia metallidurans*) also has been noted (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 lead resistance in *Escherichia coli* (*znt*A; Beard et al., 1997; Rensing et al., 1997; Rensing et al., 1998), *Staphylococcus aureus* (*cad*A), and *Pseudomonas putida* (*cad*A2) (Hynninen et al., 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, harbor lead containing nanoparticles intracellularly (Chowdhury et al., 2008; Chowdhury et al., 2011). These strains have been used to develop a bioremediation package for removal of lead from lead contaminated wastewater.

Intracellular accumulation of lead precipitates has been associated with cell morphological changes such as shortening and thickening cells of *P. aeruginosa* (Chowdhury et al., 2008), spheroidal 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 aeruiginosa*, *Bacillus subtilis subsp. subtilis* and *Bacillus amyloliquerfaciens* (Chowdhury et al., 2008; Chowdhury et al., 2011) have increased exopolysaccharide secretion associated with lead precipitation, thus providing a two-layer defense against lead 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 zinc and cadmium resistance (Turner et al., 1993; Naz et al., 2005) and the literature suggests that lead may also be sequestered intracellularly by a metallothionein (Roane, 1999). Roane (1999) observed cytoplasmic and periplasmic lead accumulation in *Bacillus megaterium* by transmission electron microscopy and postulated both efflux and metallothionein involvement in lead resistance. Cadmium localization is similar in *Desulfivibrio*

desulfuricans DSM 1926 and *Desulfococcus multivorans* DSM 2059. Cytoplasmic accumulation has been linked to a *Synechococcus* PCC 7942 metallothionein *smt*AB gene homolog involved in zinc resistance, providing more supporting evidence of metallothionein involvement (Robinson et al., 1990; Naz et al., 2005). Cadmium, zinc and copper treatment increase *smt*A and *smt*B transcript abundance and deletion mutations in the *smt*A gene, which is known to bind zinc and cadmium, results in decreased zinc resistance (Robinson et al., 1990; Turner et al., 1993). Increased metallothionein production has been correlated to lead exposure in *Bacillus cereus* (Murthy et al, 2011). *Salmonella choleraesuis* strain 4A and *Proteus penneri* strain GM10 harbor the *smt*A gene and bioaccumulate lead, suggesting probable metallothionein involvement in lead sequestration in these species (Naik, Shamim, et al., 2012c). The gene *bmtA*, encoding a related bacterial metallothionein, is detected in *Pseudomonas aeruginosa* strain WI-1, accompanied by induction of a probable *bmtA* gene product (metallothionein protein) and intracellular sequestration of lead (Naik et al., 2012b). The *bmtA* gene has been described in *Pseudomonas aeruginosa*, *Pseudomonas putida* and *Anabaena* PCC 7120 and binds zinc (Blindauer et al., 2002).

4. Leveraging lead resistance genes for lead biosensor development

4.1 lead resistance genes

Metal resistance genes are chromosomally- and plasmid-linked (Janssen et al., 2010) in the lead resistant *C. metallidurans* strain CH34 (Borremans et al., 2001; Hynninen et al., 2010; Mergeay et al., 1985). Resistance is ascribed to plasmid pMOL30, which also conveys resistance to Ag(I), Cd(II), Co(II), Hg(II), and Zn(II) (Mergeay et al., 1985; Monchy et al., 2007). The functional aspects of the lead resistance operon, *pbrUTRABCD*, have been elegantly described (Borremans et al., 2001; Chen et al., 2007; Hynninen et al., 2009; Monchy et al., 2007). 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, Julian, & Brown, 2012). An intracellular lead chaperone protein (Taghavi et al., 2009), the *pbrD* gene product, binds lead 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 lead phosphate and is sequestered in the periplasm. This leads to discontinued expression of the *pbr* operon; however, *pbrC* and *pbrD* gene product synthesis is initiated (Hynninen et al., 2009). *PbrD* may also 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 however *pbrU* is inactivated in *C. metallidurans* (Taghavi et al., 2009; Van Houdt, Monchy, Leys, & Mergeay, 2009). *PbrA* and *pbrB* are required for lead resistance; *pbrT*, *pbrC*, *pbrD* and *pbrU* are not (Hynninen et al., 2009; Taghavi et al., 2009).

C. metallidurans strain CH34 harbors additional genes that confer lead 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 *znt*A 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 can rescue mutations in complementary genes in the *C. metallidurans* strain CH34 primary lead resistance operon, *pbrUTRABCD* (Taghavi et al., 2009).

Like the *pbrR*$_2$, *cad*A, and *pbr*C_2 operons (Rmet_2302), lead resistance genes are 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) induces 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), and the lead resistance *pbr* operon is flanked by TN*4380*, a mercury transposon, suggesting potential for mobilization in the presence of lead (Monchy et al., 2007). *Delftia acidovorans* strain SPH-1 harbors *pbr* genes in a chromosomally-linked genomic island with other metal resistance determinants (Van Houdt et al., 2009). Interestingly, antibiotic resistance, which also can be linked to mobile genetic elements, has been reported to co-occur with heavy metal (including lead) resistance in many bacteria (Bharagava et al., 2014; El-Sayed, 2016; Hu and Chen, 2016; Koc, Kabatas, & Icgen, 2013; Learman et al., 2018; Matyar, 2012; Matyar et al., 2014; Pirela et al., 2014; Tomova et al., 2015). Antimicrobial resistance genes show correlation to heavy metal contamination in sediments (Ohore et al., 2018). Table 6 shows examples of multi-antibiotic & multi-heavy 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 resistance mechanisms, such as efflux pumps, that provide resistance to several pollutants.

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 lead by incorporating lead resistance genes, such as *pbrR* and *pbrA* and the luciferase reporter (*luxCDABE*) into a bacterial strain, such as *Cupriavidus metallidurans* AE2448 (formally *Alcaligenes eutrophus*; also referred to as BIOMET® Pb Biosensor or strain AE2450 (Table 7; (Geebelen et al., 2003; van der Lelie, Tibarzawa, & Corbisier, 2000). Lead specific biosensors are rare. *C. metallidurans* AE2448, which detects 0.5 μM lead 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) has successfully detected 7 – 404

mg/kg Pb (both Pb spiked and unspiked) in environmental soil samples contaminated with lead and other heavy metals, demonstrating its utility in quantifying bioavailable lead (Geebelen et al., 2003; van der Lelie et al., 2000). In addition to lead, metal specific BIOMET® biosensor constructs using *Cuprividus metallidurans* CH4 have been developed for Zn, Cd, Cr and Ni and Cu in *Cupriavidus silverii* DS185 (formally *Ralstonia silverii*) (Corbisier et al., 1994; Corbisier et al., 1999; van der Lelie et al., 2000a; Van der Lelie et al., 2000b). When *pbr*R and *pbr*A are inserted into a *Pseudomonas fluorescens* plasmid (level of detection (LOD) 0.2 μM Pb) or the chromosome (LOD 0.9 μM Pb) with the *lux* reporter, Hg, Cd, and Zn also are detected (Corbisier et al., 1999). While the biosensors use the same genetic elements, host strains and constructs are not identical, and this may account for differences in specificity.

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; J. 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; Rensing et al., 1998). *Staphylococcus aureus cadA* confers lead resistance; however results are 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 detect Hg, Cd, and Zn (Ivask, Rolova, & Kahru, 2009). *CadA Pseudmonas putida* biosensors are not inducible by lead; yet they can detect Zn (Hynninen et al., 2010). Chromosomal insertion slightly improves lead detection performance (LOD 0.3 μM versus 0.4 μM). Similar performance (Pb LOD 0.33 μM) is observed in *Staphylococcus aureus* RN4220 harboring a *cad*A*lux* constructed plasmid that also detects Cd and antimony (Sb) (Tauriainen, Karp, Chang, & Virta, 1998).

Another biosensor approach harnesses *zntA* and *zntR* (receptor), the Zn/Cd/Pb/Hg transporting ATPase, involved in heavy metal resistance (Ivask et al., 2009). This construct shows good sensitivity to lead (LOD 0.4 μM, *Pseudomonas fluorescens*) and also detects Hg, Cd, and Zn (Ivask et al., 2009). In *Escherichia coli* MG1655 harboring plasmid pZNTlux, lead detection improves 10 fold (LOD 0.03 μM); however, this biosensor also detects Cd, Zn, Hg, Co, Ni, Sb, and Cr (Riether, Dollard, & Billard, 2001; Reither et al., 2001). Zhang et al. (2017) have developed a *zntAlux* biosensor that detects 1.2-4 ng/L of lead in environmental water samples, however specificity is 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, detects Pb (LOD 0.9 μM), Zn, and Cd (Hynninen et al., 2010). Interestingly, in the *cadA1*, *cadA2*, *czcCBA1*, and *czcCBA2* deletion strain *Pseudomonas putida* KT2440.2431, lead sensor sensitivity improves 45 fold (LOD 0.02 μM), possibly because chromosomal genes are not binding available lead or, because *czc*CBA1 may be involved in lead export, more lead remains 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, can detect 4036-10469 mg/kg Pb in incinerator fly-ash as well as Zn, Cd, and Co (Corbisier, Thiry, & Diels, 1996).

5. Conclusions and future perspectives

While it has long been recognized that exposure to lead contamination is a critical stressor to microorganisms, the spatiotemporal variability of the microbial community and experimental uncertainties associated with the measurement of lead in the laboratory are the main reasons for the recommendation to the US EPA that microorganisms not be considered as an ecological endpoint in the screening level ecological risk assessment for Superfund sites (U.S. EPA, 2003). This recommendation partly inspired this literature review of relative research conducted over the past decades. Upon conclusion of this review, evidence supports that revisiting the recommendation with a focus on microbial indicators of lead contamination, could lead to the development of a framework to inform the ecological risk assessment for a contamination site (e.g., Superfund sites in the USA). To that end, a few points concluded from this review along with future research are worth discussing.

First, the current literature, including both *in vitro* and *in situ* studies, indicates that exposure to lead contamination inhibits microbial activity resulting in reduced respiration, suppressed metabolism, and reduced biomass as well as altered microbial community structure (Table 2; Table 3; de Vries et al., 2007; Xu et al., 2018; Xu et al., 2019). While most *in vitro* lead incubation studies are not long enough to reach equilibrium and represent field conditions, findings from them are generally in agreement with conclusions from long term *in situ* studies of lead and other heavy metals in terms of lead toxicity and impacts on microbial community structure and activities (Table 1). Although other physical, chemical, and biological factors in the field such as temperature, moisture, other concomitant heavy metals, pH, soil or sediment particle size distribution, organic carbon content, and vegetation types could mask microbial community shifts associated with lead contamination, the latest advancements in molecular techniques (e.g. 16S rRNA gene sequencing, functional gene microarray; metagenomic sequencing) coupled with statistical and network analyses have allowed for detailed phylogenetic analyses, functional gene identification, and discernment of the effects of lead and other heavy metals on microbial communities (Table 2; Table 3; Jacquiod et al., 2018). Even at sites where microbial communities are compositionally similar, long term heavy metal exposure results in functional differences regardless of the contamination level. Gene transfer and DNA recombination are 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 microbial community structure analysis should be developed and tested with samples representing varying spatiotemporal scales along with determination of total and bioavailable lead. Interlaboratory comparison of the resulting methodology can inform reduction of measurement uncertainties. The resulting microbial community shifts can serve as indices of lead contamination and bioavailability and inform both human health and ecological risk assessments.

Second, in apparent response to varying environmental levels of lead stress, microorganisms can initiate a series of lead resistance and adaptation mechanisms, even at a very early stage of exposure in the presence of low lead concentrations. It is well known that microbial lead resistance can be accomplished through intracellular and/or extracellular precipitation, adsorption, complexation and efflux pumps (Table 4; Table 5). Although identifying a unified set of lead resistant microorganisms among different sites can be challenging due to variable site conditions, some lead resistant bacteria such as the Gram negative *Proteobacteria* and Gram positive *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* are common and dominant in many contaminated sites. These phyla also are found in piping biofilms where microorganisms are exposed to lead contamination due to corrosion of lead pipes (White et al., 2011). Thus, another important future research area is to examine lead resistant bacteria as benchmark indictors for evaluation of lead contamination and toxicity, because microbial lead toxicity is directly dependent upon lead bioavailability. Varying levels of bioavailable lead could trigger different resistance and adaptation strategies possessed by different bacterial strains in the community. In other words, the genes bacteria use to become adapted to a specific level or range of environmentally bioavailable lead offer a means to evaluate the ecotoxicological effects at contaminated sites. Thus, quantifying the relationship between the bioavailable portion of total lead and microbial ecotoxicity and lead resistance 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.

Last but not least, the latest molecular understanding of lead resistance mechanisms has identified suites of functional genes responsible for specific lead resistance mechanisms. These resistance genes may serve as indicators of lead contamination in association with dominance of certain microbial phyla. This advanced understanding has led to the development of several lead biosensors in the realm of environmental biotechnology (Table 7). Using biosensors containing lead resistance genes may provide an indication of lead bioavailability because the microbes can sense biologically available lead (as opposed to unavailable, bound lead). In deciding which sensor to use, specificity is a key consideration because some sensors can detect several metals while a few are specific for lead. Nevertheless, the biosensor approach can be less costly to use as a screening assay and has application in lead bioavailability for ecological risk assessment. Future research should be directed to validate the effectiveness and reliability of these sensors with conventional methods for bioavailable lead detection.

In conclusion, microorganisms play an important role in lead biogeochemistry and have adapted to populate niches left available by lead sensitive microbes following contamination events. Understanding the dynamics of lead induced community shifts, to include prevalent resistance mechanisms, gives better insight into the toxicological effects of lead. Harnessing these organisms at the molecular, population, and community levels has the potential to better predict bioavailable lead levels, thereby strengthening both human and ecological risk assessments.

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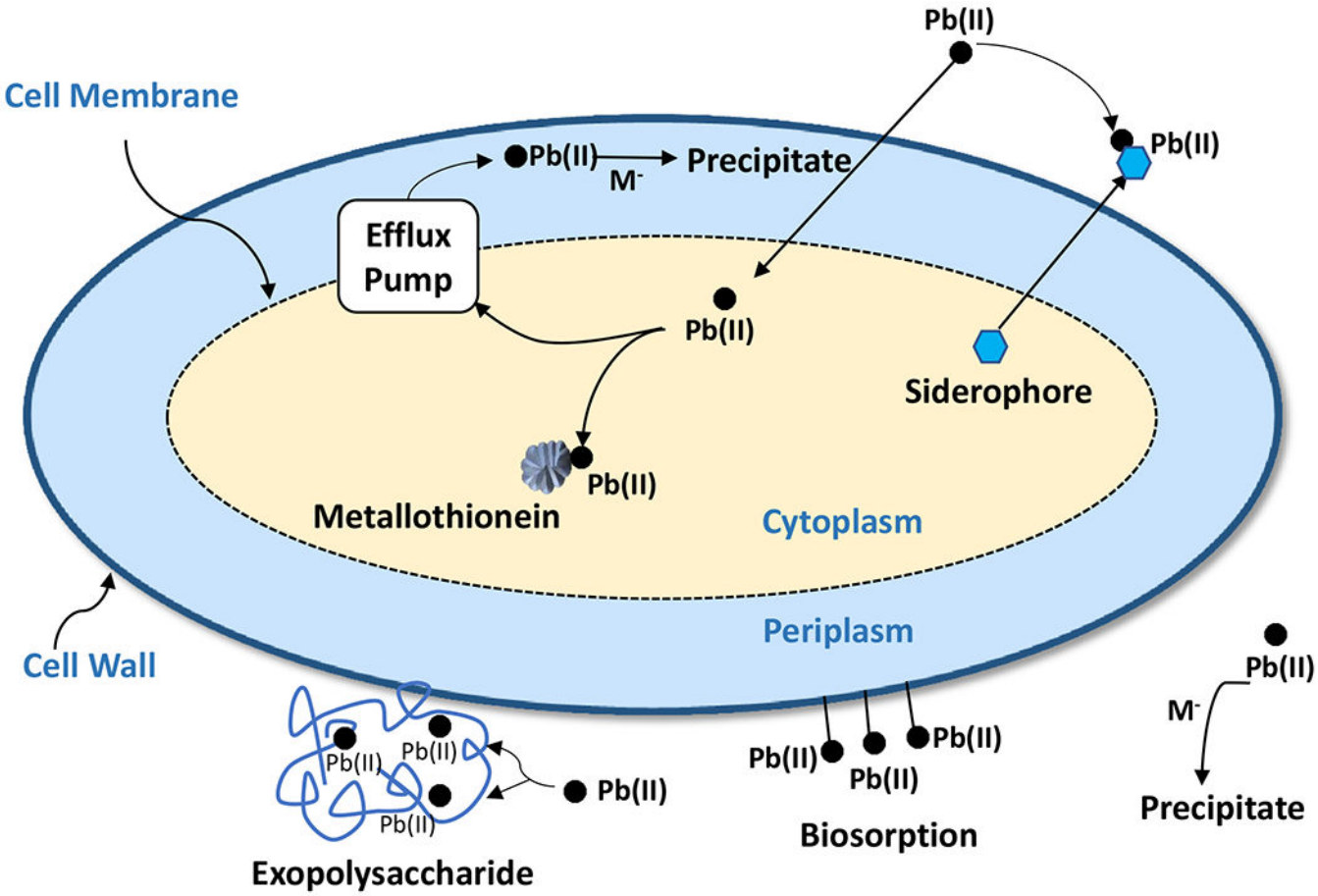

Figure 1.

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).

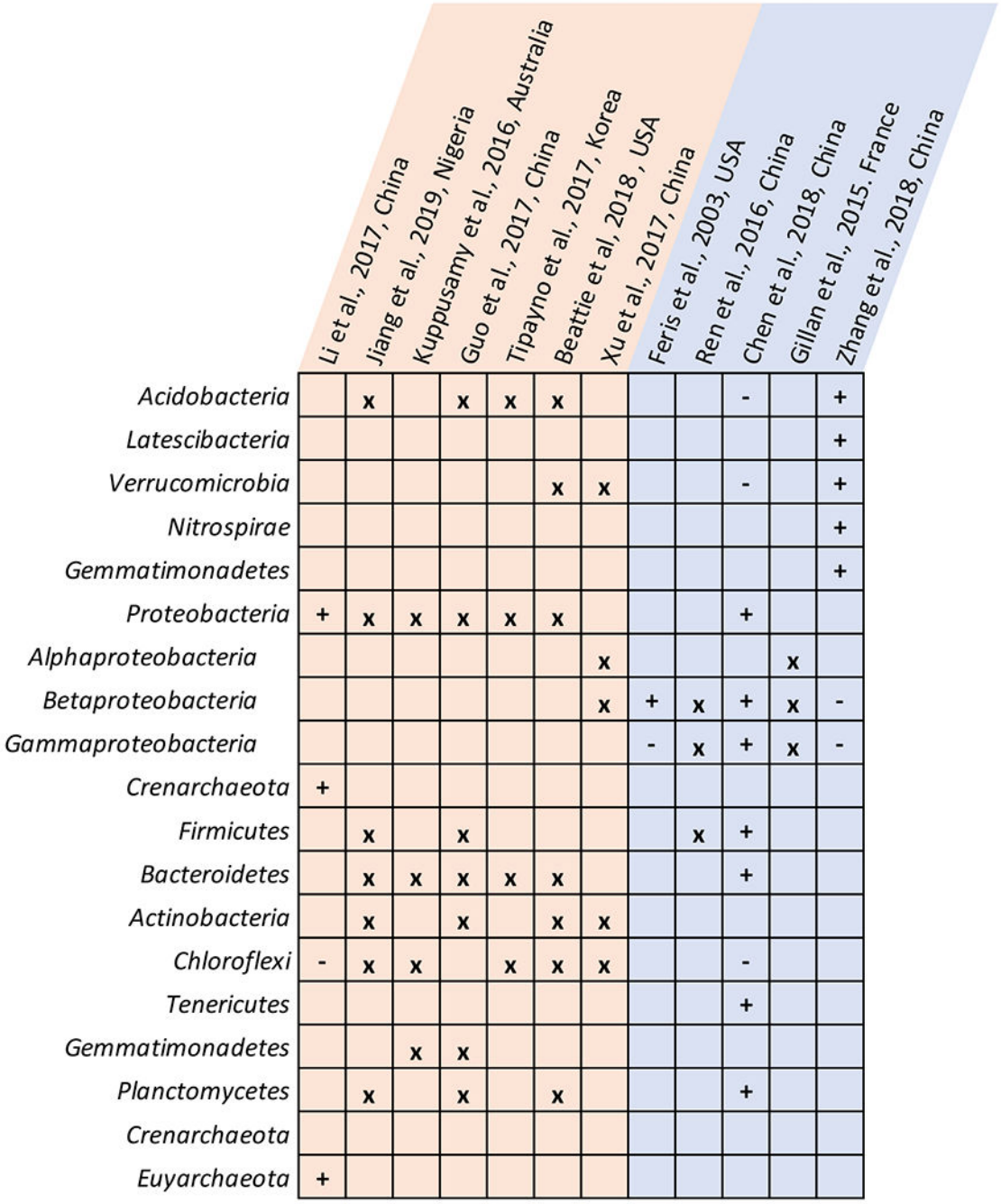

Figure 2.

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.

Table 1.

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 and 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.

Table 2.

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] *Gamma*proteobacteria (−) *Beta*proteobacteria (+) 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, 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 Férin Cd, 1.29 Cu, 13.76 Pb, 111.58 Zn, 348.55 +Al, As, Co, Cr, Fe, Mn, Ni, V	*Betaproteobacteria* *Gammaproteobacteria* *Alphaproteobacteria* *Deltaproteobacteria* Gammaproteobacteria *Betaproteobacteria* *Betaproteobacteria* *Deltaproteobacteria* *Actinobacteria*	Dominant: *Burkholderia, Rubrivivax, Leptothrix, Cupriavidus, Methylibium, Variovorax, Thauera, Azoarcus* *Pseudomonas* *Methylobacterium* *Anaeromyxobacter* Genera: (+) *Pseudomonas, Pseudoxanthomonas, Stenotrophomonas* (+) *Thiobacillus, Acidovorax, Dechloromonas, Alicycliphilus* (−) *Rubrivivax, Leptothrix* (−) *Anaeromyxobacter, Sorangium* (−) *Mycobacterium, Streptomyces*	Gillan et al., 2015

Site history	Total metal concentration (mg/kg)	Community Effects[a]		Reference
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 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

[c]BDL, Below detection limit

Table 3.

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 & smouldering 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* *Gammaproteobacteria* *Gemmatimonadetes* *Betaproteobacteria* *Actinobacteria*	Genera: (+) *Acidibacter* (+) *Blastocatella* (+) *Flavobacterium, Pedobacter* (−) *Sphingomonas* (−) *Acinetobacter*, *Pseudomonas, Mizugakiibacter*, *Rhodanobacter* (−) *Gemmatimonas* (−)*Ralstonia* (−) *Arthrobacter*	Guo, et al., 2017
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	Pb, Cd, Zn, Mg (−) Bacteria Al, Cd, Pb, and/or Zn (+) or (−) *Acidobacteria*, *Actinobacteria*, *Bacteriodetes*, *Chloroflexi*, *Plantomycetes*, *Proteobacteria*, *Verrucomicrobia* *Actinobacteria*	Genera: (+) *Streptomyces, Amycolatopsis* (+) *Flavisolibacter* (+) *Sphingomonas* (+) *Geobacter* (+) *Gemmata, Planctomyces*	Beattie et al, 2018 Beattie et al., 2017 (analytical chemistry)

Site History	Total Metal Concentration (mg/kg)	Community Effects[a]		Reference
	+Te, W, K, Mn, Ni, Fe, V, B, Cu, Co, Cr, Ti, Na	*Bacteroidetes* *Alphaproteobacteria* *Deltaproteobacteria* *Planctomycetes*		
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 Pb, 53.8-455.3 Zn, 44.6-102.6	Dominant (in descending order of abundance): *Proteobacteria, Chloroflexi, Acidobacteria, Bacteroidetes* Smelting (−) *Chloroflexi, Chlorobi* *Firmucutes* *Deltaproteobacteria* *Deltaproteobacteria* *Firmucutes* *Deltaproteobacteria* *Actinobacteria* *Deltaproteobacteria* *Deltaproteobacteria* *Deltaproteobacteria* *Deltaproteobacteria*	Genera: As(+) Bacillus As(+) Desulfatibacillum As(−) *Desulfovirga* Cd (+) *Bacillus* Cd (−) *Desulfatibacillum*, *Desulfoglaeba, Desulfovirga* Cu (+) *Streptomyces* Cu (−) *Desulfatibacillum*, *Desulfococcus,Desulfovirga* Pb (+) *Desulfatibacillum*, *Desulfovirga* Pb (−) *Desulfococcus* Ni (+) *Desulfovirga*	Tipayno et al., 2017

[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 4.

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

Isolation source	Pb(II) media	Phylum[a]	Genus/Species	Reference
Exopolysacchride (EPS) production				
Coast	$Pb(CH_3COO)_2$ 100ppm	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	De et al., 2008
Lead battery manufacturing plant	Pb(NO3)2 1.6 mM	*Gammaproteobacteria*	*Enterobacter cloacae*	Naik et al., 2012
-	$Pb(C_2H_3O_2)_2$ 1.3 – 13 mg/L	*Gammaproteobacteria*	*Marinobacter* sp.	Bhaskar & Bhosle et al., 2006
-	-	*Betaproteobacteria*	*Burkholderia cepacia* (formerly *Pseudomonas cepacia*)	Nelson et al., 1995
-	$Pb(NO_3)_2$ 1mM	*Gammaproteobacteria*	*Citrobacter* sp.	Macaskie and 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 and Duby, 2011
-	$Pb(NO_3)_2$	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	O'Brien et al., 2014
Surface biosorption				
Hospital sewage	$PbCI_2$	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	Chang et al., 1997
Soil	-	*Gammaproteobacteria*	*Pseudomonas aeruginosa*	Karimpour et al., 2018

[a] *Proteobacteria* are listed by class if available

Table 5.

Bacteria species with intracellular Pb accumulation: precipitation or metallothionein binding

Isolation source	Pb (II) media	Phylum[a]	Species	Precipitate/Metallo-thionein 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 nano-particles (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., 2017
-	$Pb(NO_3)_2$ 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 & PbSO4	Li et al., 2016
Metal mine soil	$Pb(NO_3)_2$ 0.1 mM	*Firmicutes*	*Bacillus megaterium*	Mineral unidentified	Roane, 1998
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., 2018
Estuary	$Pb(NO_3)_2$ 0.6 mM	*Gammaproteobacteria*	*Pseudomonas* sp.	*bmtA*	Naik et al., 2012
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

Table 6.

Examples of multi-antibiotic and multi-heavy metal resistance in bacteria.

Source	Resistance	Phylum	Species	Reference
Industrial waste water	14 antibiotics & 5 heavy metals	*Gammaproteobacteria*	*Acinetobacter baumanni*	El-Sayad, 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.	Learman et al., 2018
Coast	16 antibiotics & 5 heavy metals	-	Gram negative bacteria	Matyar, 2012
River	4 antibiotics & 5 heavy metals	-	Gram negative bacteria	Matyar et al., 2014

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	***pbrR*POΔ*pbrA*::*lux*[d]	0.5	N.D.[c]	Corbisier et al., 1999
Pseudomonas fluorescens OS8	pDN***pbrR*P*pbrA****lux*	0.9	Hg, Cd, Zn	Ivask et al., 2009
Pseudomonas fluorescens OS8	Kn***pbrR*P*pbrA****lux*	0.2	Hg, Cd, Zn	Ivask et al., 2009
Pseudomonas fluorescens OS*8*	pDN***zntR*P*zntA****lux*	0.4	Hg, Cd, Zn	Ivask et al., 2009
Pseudomonas fluorescens OS*8*	pDN***cadR*P*cadA****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**P*czc1****lux*	0.9	Zn, Cd	Hynninen et al., 2010
Pseudomonas putida KT2440.2431[g]	pDN**P*czc1****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
Environmental samples				
BIOMET® Pb biosensor	***pbrR*P*pbrA****lux*	7 – 404 mg/kg Soil +/− Pb2+ amendment[h]		Geebelen et al., 2003
Cupriavidus metallidurans	**zntAP***lux*	1.2 – 4 ng/L Water	N.D.	Zhang et al., 2017
Alcaligenes eutrophus[a] AE1433	pMOL30::Tn*4431*[b]	4036-10469 mg/kg Incinerator fly-ash	Zn, Cd, Co	Corbisier et al., 1996

[a] *Cupriavidus metallidurans (Betaproteobacteria);* formally *Alcaligenes eutrophus, Wautersia metallidurans, Ralstonia metallidurans)*

[b] Tn*4431* 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 et al., 2007;)

[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 pDN**P*cadA1****lux*.

[h] BIOMET assay determined Pb(II) concentration