34 Cadmium and Lead Tolerance Mechanisms in Bacteria and the Role of Halotolerant and Moderately Halophilic Bacteria in Their Remediation

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CONTENTS

ABSTRACT

In this chapter, the general mechanisms of heavy metal resistance in bacteria and the potential of halophilic bacteria in bioremediation with special reference to cadmium and lead are reviewed. In the present scenario, we need strains with high metal resistance and removal capacity in varying salinities. It is a well-known fact that the constituents of bacterial growth media and also the salt will react with cadmium and lead. The toxicity of a metal to bacteria strongly depends on its bioavailability rather than its concentration. At higher concentrations, lead tends to precipitate more in the media. So when conducting metal reduction experiments, care should be given to constituents of media and the concentration of the metal tested. Though there were a number of studies dealing with the interference of culture medium components and salinity with metals, there is still a long way to go.

34.1 INTRODUCTION

Anthropogenic activities release many toxic elements and compounds in quantities that exceed the carrying capacity of the environment. They impair the self-cleansing mechanisms present in nature and have become a source of pollution. The toxic chemicals released to the environment tend to bioaccumulate in the living organisms. Metals/metalloids are a major class of these compounds that cause undesirable effects in living systems from micro- to macrobiota including human beings. Even micronutrients such as calcium, cobalt, chromium, copper, iron, magnesium, manganese, nickel, potassium, sodium, and zinc are toxic to living systems at higher concentrations (Nies, 1999). Based on the disability-adjusted life year (DALY) estimates, deaths from chemical pollution of soil, water, and air are increasing (GBD, 1990; World's Worst Pollution Problems, 2015). In the "Priority List of Hazardous Substances" prepared by ATSDR (2015), lead and cadmium are in the second position and seventh position, respectively.

The International Agency for Research on Cancer has classified cadmium and cadmium compounds as a Group 1 carcinogen and lead compounds as Group 2 carcinogens.

Globally, 26 million people are at risk of exposure to lead, with an estimated burden of disease of 9 million DALYs. According to the estimates by Pure Earth, 5 million people are at risk of exposure to cadmium globally,

with an estimated burden of disease of 250,000 DALYs. As of 2015, the Toxic Site Identification Program has identified over 150 sites around the world where exposure to cadmium threatens the health of the population (World's Worst Pollution Problems, 2015). Many studies from all over the world have reported the toxic effects of lead and cadmium (Haefliger et al., 2009; Checconi et al., 2013; Choi and Han, 2015; Han et al., 2015).

Chemical precipitation, oxidation/reduction, filtration, ion exchange, reverse osmosis, membrane technology, evaporation, and electrochemical treatment are the major physicochemical processes used for the remediation of heavy metals from wastewater. Physical separation methods become ineffective in the separation of metal salts because most of the heavy metal salts are water soluble (Hussein et al., 2004). The chemical methods are also not better when the heavy metal concentration is less than 100 mg/L (Ahluwalia and Goyal, 2007). Bioremediation is an effective and economical alternative when the physicochemical methods fail to remove small concentrations of heavy metals from wastewater.

34.2   BIOREMEDIATION

Bioremediation involves a variety of processes such as biosorption, bioaccumulation, and biotransformation, and it can be done by using various biological means such as plants, algae, fungi, yeast, and bacteria. Because of their small size, short regeneration time, and ability to utilize multiple substrates for growth, bacteria are a favorite choice for the bioremediation of toxic metals.

The industries that manufacture chemicals such as pesticides, pharmaceuticals, and herbicides and oil and gas recovery processes produce wastewaters with salinity fluctuating from time to time. Conventional microbiological treatment processes do not function effectively at these salinity variations because of the inadaptability of microbes to varying salt concentrations. As the extreme halophiles—haloarchaea—that thrive at high salinities cannot withstand low salinity, moderately halophilic or halotolerant bacteria assume significance.

According to Kushner (1978), moderate halophiles are organisms growing optimally between 0.5 and 2.5 M salt. Bacteria able to grow in the absence of salt as well as in the presence of relatively high salt concentrations are designated as halotolerant (or extremely halotolerant if growth extends above 2.5 M). Therefore, the use of moderately halophilic or halotolerant bacteria is a better option (Oren et al., 1992, 1993) for bioremediation in saline environments.

34.3   MECHANISMS OF METAL RESISTANCE

Cadmium and lead toxicity in microorganisms involves thiol binding and protein denaturation, interaction with calcium metabolism and membrane damage, interaction with zinc metabolism, or loss of a protective function, inhibition of enzyme activity, disruption of membrane functions, and oxidative phosphorylation as well as alterations of osmotic balance (Vallee and Ulmer, 1972; Nies, 1999; Bruins et al., 2000; Ahemad, 2012).

There are different mechanisms for bacteria to resist the heavy metals. They can prevent the entry of toxic metals into the cell or accumulate them inside the cell in a form that is not toxic to the cell or expel the metals outside the cell after converting their chemical composition. The general mechanisms of heavy metal resistance in bacteria are summarized in Figure 34.1.
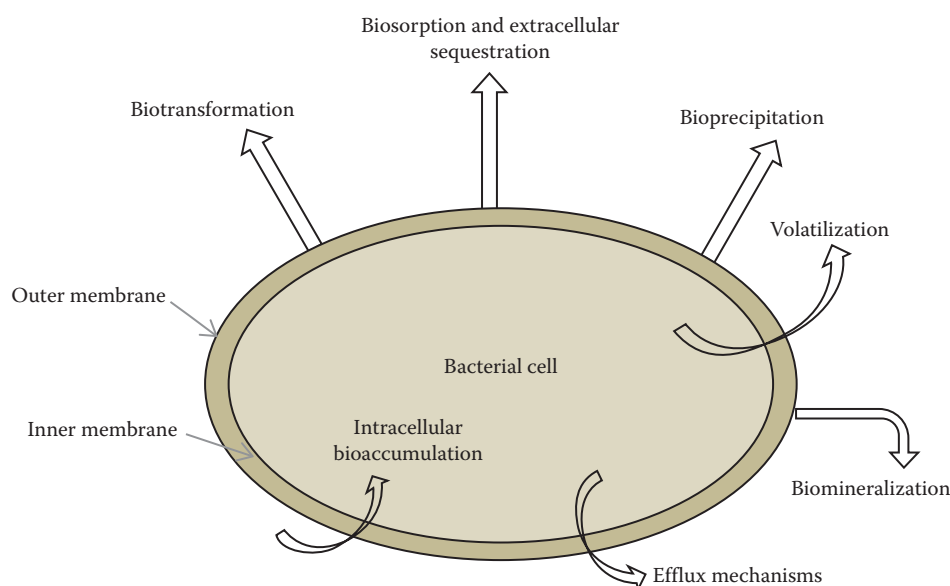

FIGURE 34.1   General mechanisms of heavy metal resistance in bacteria.

34.3.1 Biosorption

Biosorption is the phenomenon of removing heavy metals from aqueous solutions applying inactive or dead biomass (Volesky, 2001; Ilamathi et al., 2014). It is an energy-independent phenomenon. In this process, metal ions can interact on bacterial surface via physical or chemical mechanisms. Aryal and Liakopoulou-Kyriakides (2015) have summarized the effects of different external factors such as pH, contact time, temperature, concentration of biomass and metal ions, and the nature of aqueous environment in the degree of biosorption efficiency of bacterial cells.

34.3.2 Cell Wall and Extracellular Polymeric Substances

The metal homeostasis achieved by preventing the entry of metals inside the cell is known as surface biosorption or extracellular sequestration. In gram-negative bacteria lipo-polysaccharides and in gram-positive bacteria, peptidoglycan together with teichoic and teichuronic acids acts as the main binding site for heavy metals. Many studies show that cell envelope is a major barrier for lead. It prevents the entry of lead inside the cell (Massadeh et al., 2005; Kim et al., 2007; Issazadeh et al., 2011; Syed and Chinthala, 2015).

Exopolymers or slime layers are composed of carbohydrates, polysaccharides, and sometimes nucleic acids and fatty acids. Extracellular polymeric substance (EPS) performs a variety of functions such as protection against desiccation, phagocytosis, and parasitism and helps in the formation of biofilm, (Maier et al., 2006). EPSs are generally negatively charged, and consequently, the efficiency of metal–exopolymer binding is pH dependent. Studies on the metal-binding behavior of EPSs revealed their great ability to complex heavy metals through various mechanisms, which include proton exchange and microprecipitation of metals (Comte et al., 2008; Fang et al., 2010). Many reports establish the importance of EPS molecules in metal removal (Arias et al., 2003; Mata et al., 2006; De et al., 2008).

Cadmium and lead are biosorbed differently by different bacteria. The bacterial cell walls are primarily made up of polysaccharides, proteins, and lipids. Polysaccharides have ion-exchange properties. These basic units host a variety of functional groups such as amino, carboxylic, sulfhydryl, phosphate, and thiol groups, which differ in their affinity and specificity for metal binding. Biosorption of heavy metals can be influenced by the composition of EPS, especially by the presence of sulfates and uronic acid (Iyer et al., 2005). The uronic acids and sulfates form gels in the presence of metal ions and thus help in removing them from polluted environments.

The metal-binding capacity of a polymer depends upon the quantity and accessibility of charged groups to the ions (Sutherland, 1994; Arias et al. 2003). *Halomonas maura* strain S-30—a diazotrophic bacterium—produces mauran, with an EPS contributing to the formation of biofilms and binding of some heavy metals. High sulfate and uronic acid contents make mauran an excellent biosorbent for heavy metals especially lead. Native mauran showed high binding capacity to Pb, while deacetylated mauran had significant affinities for both Ag and Pb (Arias et al., 2003). It may be because acetyls bring more electron-donating groups into the vicinity of the binding site, thus allowing the larger Pb ions to bind more strongly (Geddie and Sutherland, 1993, 1994; Arias et al., 2003). The removal of acetyl groups leaves the binding sites more accessible to the cations. However, anionic polysaccharides prefer on the whole to bind cations with large ionic radii (Geddie and Sutherland, 1993).

Mata et al. (2006) demonstrated that EPSs from *Halomonas ventosae* strain Al12T had a significant affinity for lead and that from strains *H. ventosae* Al16 *Halomonas anticariensis* FP35T and FP36 bound copper most efficiently. All the polymers showed a lower affinity for cobalt.

EPS solutions from *H. maura*, *H. ventosae*, and *H. anticariensis* did not produce stable gels in the presence of the metal salts, but they did bind several metals with considerable efficiency. Ion uptake by EPS from all the strains started almost immediately and increased with time.

Enterobacter cloacae P2B isolated from the effluent of lead battery manufacturing company was found to increase its EPS production when exposed to lead nitrate in Tris-buffered minimal medium (Naik et al., 2012). Fourier transform infrared spectroscopy (FTIR) of this EPS revealed carboxyl, hydroxyl, and amide groups along with glucuronic acid. It also showed the presence of several neutral sugars such as rhamnose, arabinose, xylose, mannose, galactose, and glucose contributing to lead-binding hydroxyl groups.

Attenuated total reflectance FTIR spectroscopy suggested that sulfhydryl group (-SH) within the EPS of *Pseudomonas aeruginosa* JP-11 plays a major role in the binding of cadmium (Chakraborty and Das, 2014). A novel EPS called EPS-R produced by a slightly halophilic marine bacterium *Hahella chejuensis* showed specific emulsifying capacity higher than that of commercial polysaccharides and stability over a wide range of temperatures, pH, salinities, etc. (Ko et al., 2000).

34.3.3 Biosurfactants

Biosurfactants being amphiphiles consist of two parts—a polar (hydrophilic) moiety and nonpolar (hydrophobic) group that are produced by microorganisms. A hydrophilic group consists of mono-, oligo-, or polysaccharides, peptides, or proteins, and a hydrophobic moiety usually contains saturated, unsaturated, and hydroxylated fatty acids or fatty alcohols (Lang, 2002). A characteristic feature of biosurfactants is a hydrophilic–lipophilic balance that specifies the portion of hydrophilic and hydrophobic constituents in surface-active substances. Pacwa-Płociniczak et al. (2011) summarized the current status of the environmental applications of biosurfactants. Glycolipid (rhamnolipids), fatty acids, phospholipids and neutral lipids (phosphatidylethanolamine), and lipopeptides (surfactin) are the major groups and classes (in parenthesis) of

biosurfactants produced by bacteria that are used for the bioremediation of metals. *Pseudomonas* sp., *Acinetobacter* sp., *Rhodococcus erythropolis*, and *Bacillus subtilis* are the major bacteria reported to produce these surfactants (Jennema et al., 1983; Appanna et al., 1995; Herman et al., 1995).

Biosurfactants remediate metals by forming complexes with them. Metal–anionic biosurfactant bonds are stronger than metal–soil bonding, and eventually, the metal–biosurfactant complex gets desorbed to the soil solution due to the lowering of the interfacial tension. The cationic biosurfactants can replace some of the cationic metal ions by competition, but it is not possible in the case of all negatively charged surfaces (ion exchange). However, metal ions in the soil surfaces can be removed through the biosurfactant micelles.

The polar head groups of micelles can bind metals that mobilize the metals in water (Deziel et al., 1996; Singh and Cameotra, 2004; Juwarkar et al., 2007; Asci et al., 2008).

Juwarkar et al. (2008) reported the use of dirhamnolipid biosurfactant produced by *P. aeruginosa* BS2 for the mobilization of metals from multimetal-contaminated soil. The dirhamnolipid selectively removed heavy metals from soil in the order of Cd = Cr > Pb = Cu > Ni. The use of rhamnolipid together with inorganic compounds (e.g., NaOH) was found to increase the efficiency of the removal of copper and nickel from sediments (Dahrazma and Mulligan, 2007). The addition of OH– group solubilizes the naturally existing organic fraction of the metals and thus increases their availability to the surfactant. Wang and Mulligan (2004) proved that biosurfactant-foam technology is more efficient in the removal of Cd and Ni from sandy soil than the rhamnolipid solution. The rate of heavy metal removal from soil strongly depends on its chemical composition.

The removal of heavy metals by biosurfactant produced by marine bacterium was also reported (Das et al., 2009). The study revealed that tested anionic biosurfactant was able to bind the metal ions and form insoluble precipitate. The efficiency of the removal of Pb and Cd depends on the concentrations of metals and biosurfactants.

34.3.4 Efflux Systems

Efflux systems protect the cell from harmful metals by transporting the metal cations from inside the cell through the cell membrane and thus excluding them. The energy-requiring efflux systems are one of the most effective resistance mechanisms present in bacteria against heavy metals (Bruins et al., 2000).

According to Nies (1995) and Nies and Silver (1989), resistance to cadmium in bacteria is based on cadmium efflux. The efflux of Cd in bacteria is facilitated by P-type ATPases, CBA transporters, and cation-diffusion facilitator (CDF) chemiosmotic transporters. Magnesium and/or manganese uptake systems are also responsible for the uptake of Cd (Tynecka et al., 1981; Laddaga et al., 1985). In gram-negative bacteria, cadmium seems to be detoxified by resistance–nodulation–cell division (RND)-driven systems like Czc, which is mainly a zinc exporter, and Ncc, which is mainly a nickel exporter

(Schmidt and Schlegel, 1994). The product of genes *czcA*, *czcB*, and *czcC* forms the membrane-bound cation efflux protein complex *czcABC* that includes three subunits: CzcA, a cation/proton antiporter (in the cytoplasmic membrane); CzcB, a membrane fusion protein (in the periplasm); and CzcC, an outer membrane protein. They are responsible for the transport of the cations across the cytoplasmic membrane, the periplasmic space, and the outer membrane, thus resulting in the decrease of intracellular cations. In gram-positive bacteria, the primary resistance mechanism is by the cadmium-exporting P-type ATPase, that is, the CadA pump from *Staphylococcus aureus* (Nucifora et al., 1989). This protein was the member of a subfamily of heavy metal P-type ATPases, and all the copper, lead, and zinc transporters found later are related to this protein. Liu et al. (1997) reported that CadA-like proteins mediate cadmium resistance in other gram-positive bacteria like *Bacillus firmus* and *Listeria monocytogenes*.

Eleven distinct *Staphylococcus*, *Micrococcus*, and *Halobacillus* genera carrying the *cadA* gene were reported by Oger et al. (2003). This *cadA* determinant was mostly plasmid borne in the *Staphylococcus* genus, and IS257 sequences, which are known to participate in antibiotic resistance gene dissemination in *S. aureus*, were found to be located near to the *cadA* gene in 16/31 cadmium-resistant *Staphylococcus* strains and one *Micrococcus* strain. This suggests that IS257 has also contributed to the dissemination of the cadA resistance gene among staphylococci. All of the *Staphylococcus* and *Micrococcus* species studied carried the *cadA* gene on plasmids, whereas the *cadA* gene is chromosomal in members of the *Bacillus* (MSA 235) and *Halobacillus* (MSA 234) genera.

Studies on Cd/Zn chemiosmotic efflux transporter (CzcCBA1) in Cd-tolerant *Pseudomonas putida* KT2440 by Gibbons et al. (2011) proved that heavy metal cation efflux mechanisms facilitate shorter lag phases in the presence of metals and the maintenance and expression of tolerance genes carry quantifiable energetic costs and benefits. In *Pseudomonas stutzeri*, numerous genes responsible for resistance to several metals (As, Cu, Hg, Cr, Cd, and Zn) were identified (Li et al., 2012). Transmission electron microscopic (TEM) analysis of two cadmium-resistant bacteria CdRB1 revealed that it actively accumulated cadmium in the cytoplasm (Dabhi et al., 2013). Chakraborty and Das (2014) demonstrated that the cadmium-resistant gene expression increases on Cd stress up to the tolerance level, but optimum pH and salinity are the crucial factors for the proper functioning of cadmium-resistant gene.

Pb^{2+} resistance also involves P-type ATPases. Hynninen et al. (2009) confirmed that lead resistance in *C. metallidurans* is achieved through the cooperation of the Zn/Cd/Pb-translocating ATPase PbrA and the undecaprenyl pyrophosphate phosphatase PbrB. While PbrA nonspecifically exported Pb, Zn, and Cd, a specific increase in lead resistance was observed when PbrA and PbrB were coexpressed. As a model of action for PbrA and PbrB, they proposed a mechanism where Pb is exported from the cytoplasm by PbrA and then sequestered as a phosphate salt with the inorganic

phosphate produced by PbrB. Similar operons containing genes for heavy metal–translocating ATPases and phosphatases were found in several different bacterial species, suggesting that lead detoxification through active efflux and sequestration is a common lead resistance mechanism.

The scanning electron microscopy (SEM)-EDX and HR-TEM-EDX techniques revealed that the bacterium *Klebsiella* sp. 3S1 largely adsorbed lead on the entire cell surface and lead accumulated within the cytoplasm also, which indicated the presence of bioaccumulation mechanism (Munoz et al., 2015).

34.3.5 SIDEROPHORES

Siderophores are low-molecular-weight, high-affinity iron (Fe)-chelating organic compounds secreted by various microorganisms. They are excreted by microbes in iron-deficient environments. Siderophore–Fe complex formation is influenced by pH. Siderophores were reported to interact with other metals and in fact there is a competition for siderophore-binding sites between iron and other free protons and metal ions such as Cd^{2+}, Cu^{2+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} (Albrecht-Gary and Crumbliss, 1998; Schalk et al., 2011; Saha et al., 2012) and Mn^{3+}, Co^{3+}, and Al^{3+} and actinides such as Th^{4+}, U^{4+}, and Pu^{4+} (Peterson et al., 2004). Pyoverdine siderophores—defining the characteristic for *Pseudomonas*—were produced by *P. aeruginosa* 4EA and endophytic *P. putida* KNP9. Both were reported to complex Pb (Tripathi et al., 2005; Naik and Dubey, 2011). The pyoverdines produced by the plant growth-promoting strain *P. putida* KNP9 were found to reduce the concentration of Pb in mung bean roots and shoots (Tripathi et al., 2005). The presence of Pb doubled the production of siderophores in *P. aeruginosa* 4EA (Naik and Dubey, 2011). The siderophore pyochelin produced by *P. aeruginosa* PAO1 showed higher affinity toward Pb than pyoverdine (Braud et al., 2010). Braud et al. (2009) reported that though the transporter FpvAI has broad metal specificity at the binding stage, it is highly selective for Fe^{3+} only during the uptake process.

Homann et al. (2009) have isolated a suite of amphiphilic siderophores, that is, loihichelins A–F, from the cultures of the marine heterotrophic Mn(II)-oxidizing bacterium *Halomonas* sp. LOB-5. They suggested that these siderophores may have a role in sequestering Fe(III) released during basaltic rock weathering and in the promotion of Mn(II) and Fe(II) oxidation. Sinha and Mukherjee (2008) reported Cd-induced siderophore production and accumulation of Cd by *P. aeruginosa* KUCd1 in a culture medium containing cadmium.

34.3.6 BIOMINERALIZATION

Biomineralization is the process by which some halophilic bacteria synthesize inorganic solids resulting in organic–inorganic hybrids. The process depends mainly on the organism and factors such as pH and temperature. Many products of biomineralization are capable of binding environmental contaminants and removing them from wastewaters.

The moderately halophilic bacterium *Halomonas halophila* mineralizes calcium carbonate in the calcite polymorph. Rothenstein et al. (2012) studied the biomineralization process in the presence of zinc ions as a toxic model contaminant. The medium used for the experiments contained 3% sea salts. The results showed that *H. halophila* can adapt to zinc-contaminated medium, maintaining the ability for the biomineralization of calcium carbonate. The growth of the bacterium was not much affected by the presence of zinc, and it accumulated zinc ions on its cell surface causing a depletion of zinc in the medium. They also confirmed that Zn ions influence the biomineralization process. In the presence of zinc, the polymorphs monohydrocalcite and vaterite were mineralized, instead of calcite that was synthesized in zinc-free medium. That is, zinc influenced in the modification of the synthesized calcium carbonate polymorph. In addition, the shape of the mineralized inorganic material is changing through the presence of zinc ions. Furthermore, the moderately halophilic bacterium *H. halophila* can be applied for the decontamination of zinc from aqueous solutions.

Carbonate precipitation in a medium containing artificial sea salt of 7.5% (w/v) mediated by moderately halophilic bacteria *Chromohalobacter marismortui* was reported by Rivadeneyra et al. (2010).

34.3.7 BIOSYNTHESIS OF NANOPARTICLES

Nanoparticles (NPs) are defined as materials that are less than 100 nm in size. Biosynthesis of lead and cadmium NPs is reported by various organisms like bacteria, yeast, and fungi and also by various leaf extracts (Ahmad et al., 2002; Kowshik et al., 2002a,b; Prasad et al., in press). The ability of the bacteria to produce NPs is beneficial in bioremediation as well as it provides a less toxic and precise method for large-scale synthesis of these highly beneficial particles. The importance of this emerging field of study is evident from numerous reviews published in the past 5–6 years (Sinha et al., 2009; Arya, 2010; Ramezani et al., 2010; Talebi et al., 2010; Li et al., 2011; Habeeb, 2013; Ingale and Chaudhari, 2013; Hamidi et al., 2014; Iravani, 2014; Malik et al., 2014; Pantidos and Horsfall, 2014; Jacob et al., 2015)

Cysteine desulfhydrase (C-S-lyase)—an intracellular enzyme located in the cytoplasm—was reported to be an important factor in the biosynthesis of metal sulfide NPs (Wang et al., 2001). Bai et al. (2009) found out that the synthesis of CdS NPs in *R. sphaeroides* was mediated by C-S-lyase. According to Kang et al. (2008), NPs synthesized by microorganisms were stabilized by peptides such as phytochelatins, thus preventing aggregation.

Bacillus megaterium synthesized Ag, Pb, and Cd NPs when grown aerobically with the solutions of metal salts. The NPs were accumulated on the surface of the cell wall of the bacteria with particle size in the range of 10–20 nm (Prakash et al., 2010). The biomass of *Bacillus licheniformis* MTCC 9555 when challenged with cadmium chloride and sodium sulfide was found to produce CdS NPs (5.1 ± 0.5 nm) (Tripathi et al., 2014). Shivashankarappa and Sanjay (2015) studied the

effect of various ratios of $CdCl_2$ and Na_2S on CdS NP formation by *B. licheniformis*. They also demonstrated the antimicrobial activity of CdS NPs on food-borne bacteria such as *Escherichia coli*, *B. licheniformis*, *P. aeruginosa*, *Bacillus cereus*, and *S. aureus* and fungi such as *Fusarium oxysporum*, *Aspergillus flavus*, and *Penicillium expansum*. The size of CdS varied between 20 and 40 nm. The CdS NP ratio of 4:1, that is, the ratio between $CdCl_2$ and Na_2S at a concentration of 40 mg/ml, showed the highest zone of inhibition in *P. aeruginosa* and *A. flavus* (Shivashankarappa and Sanjay, 2015).

The marine bacterium *Idiomarina* sp. PR58–8 was reported to synthesize silver NPs intracellularly (Seshadri et al., 2012). Holmes et al. (1997) have reported that CdS particles synthesized by *K. pneumoniae* (>5 nm in diameter) on its outer cell wall grow continuously and some reached the size >200 nm, which are known as Q particles. *E. coli* was found to synthesize intracellular cadmium sulfide (CdS) nanocrystals (2–5 nm) when incubated with cadmium chloride and sodium sulfide (Sweeney et al., 2004). Immobilized photosynthetic bacteria *Rhodobacter sphaeroides* produce CdS at room temperature with a single-step process. CdS NPs formed intracellularly were then transported into extracellular solution. The size of sulfide NPs synthesized was found to vary with culture time—2.3–36.8 nm from 36 to 48 h (Bai et al., 2009). They found out that the synthesis of NPs was mediated by cysteine desulfhydrase (C-S-lyase)—an intracellular enzyme located in the cytoplasm.

Desulfotomaculum sp. produced 13 nm diameter PbS NPs (Jun et al., 2007). *Enterobacter* sp. that is able to synthesize lead oxide (PbO) NPs within the periplasmic space and a virulent strain of *B. anthracis* that is able to synthesize lead sulfide (PbS) NPs extracellularly were reported by El-Shanshoury et al. (2012a).

Rapid and low-cost biosynthesis of CdS using the culture supernatants of *E. coli* ATCC 8739, *B. subtilis* ATCC 6633, and *Lactobacillus acidophilus* DSMZ 20079T was reported by El-Shanshoury et al. (2012b). The sizes of the particles were found to vary between 2.5 and 5.5 nm. Seshadri et al. (2011) have reported the intracellular synthesis of stable lead sulfide NPs by the marine yeast *Rhodosporidium diobovatum*. Singha and Nara (2013) synthesized PbS NPs using the supernatants of two bacterial strains (NS2 and NS6) isolated from the heavy metal–rich soil samples of Allahabad and Kanpur. Extracellular PbS NPs were synthesized when supernatant was challenged with lead chloride and calcium sulfate salts. The enzymes involved in metal ion reduction were present only in the supernatant (Singha and Nara, 2013).

34.4 CADMIUM/LEAD RESISTANCE IN SALINE MEDIUM BY HALOTOLERANT/ MODERATELY HALOPHILIC BACTERIA

Hahne and Kroontje (1973) have reported that cadmium and lead [Cd(II) and Pb(II)] react with Cl⁻ to form a variety of coordination complexes, depending on the concentration of Cl⁻. The MCl^+ species of Cd and Pb appear at chloride concentrations above 10^{-3} M (35 ppm), and MCl_2 complexes occur above 10^{-2} M (350 ppm Cl⁻). The respective MCl_3^- and MCl_4^{2-} species become important above 10^{-1} M Cl⁻ (3500 ppm). Babich and Stotzky (1982) investigated the effect of seawater on Cd toxicity to nonmarine fungi and found that the toxicity decreased as the concentration of chloride (Cl⁻) or of seawater increased, indicating the lower toxicity of Cd–Cl complexes than that of Cd^{2+}.

Onishi et al. (1984) reported a decrease in the toxicity of cadmium in a moderately halophilic *Pseudomonas* sp. with an increase in NaCl concentration from 1 to 3 M. According to them, the cadmium tolerance of 41 strains of halophilic bacteria differed in their salt requirement and cadmium tolerance. They also showed that Cd toxicity was apparently enhanced by $NaNO_3$ and Na_2SO_4. The toxicity was measured as cell death, and accommodation to Cd required an extended lag time that varied with both the concentration of Cd and that of salts.

Garcia et al. (1987) studied 58 strains of the moderate halophilic bacteria *Vibrio costicola*, including both culture collection strains and freshly isolated strains from solar salterns, regarding their susceptibility to 10 heavy metals in basal medium with 5% and 15% salt. All strains were sensitive to cadmium, copper, silver, zinc, and mercury. Mercury showed the highest toxicity even at the lowest concentration tested. The isolates showed tolerance in the following pattern, Pb > Ni > Cr > As > Co. The majority of strains (96.4%) were multimetal tolerant, with three different metal ion tolerances as the major pattern. But no significant differences were observed for the MIC values for each individual strain when they were tested in media with 5% or 15% salts.

One of the earlier extensive works on metal-tolerant moderately halophilic eubacteria is that by Nieto et al. (1989). They studied the tolerance of moderate halophiles from culture collection and fresh isolates to 10 heavy metal ions. The tested isolates belonged to different taxonomic groups, namely, *Deleya halophila*, *Acinetobacter* sp., *Flavobacterium* sp., and gram-positive cocci (*Marinococcus*, *Sporosarcina*, *Micrococcus*, and *Staphylococcus*). All collection strains were sensitive to silver, mercury, and zinc and tolerant of lead, while they differed in their response to arsenate, cadmium, chromium, and copper. They reported variations in metal resistance pattern among strains irrespective of their taxonomic group. Mercury, silver, and zinc were reported to be the most toxic metals against the tested isolates, while arsenate was the least toxic. Two genera, namely, *Acinetobacter* and *Flavobacterium*, were found to include the most heavy metal–tolerant and heavy metal–sensitive strains, respectively. Lowering the salinity enhanced sensitivity to cadmium and, in some cases, to cobalt and copper. This may be either due to the osmotic changes in the cellular level induced by salinity resulting in a higher availability of toxic ions to bacteria or due to the formation of complex metal species that exert higher toxicities than free metal cations. However, increasing the salinity resulted in only a slight decrease in cadmium, copper, and nickel toxicities. However, zinc showed no reduction in toxicity with an increase in salinity.

In the study conducted by Gaballa et al. (2003), the moderately halophilic bacterium *Staphylococcus* sp. exhibited maximum resistance to nickel (Ni) in halophilic medium. Cd and Cu ions showed the highest toxic effect on the strain when combined in nontoxic levels with Ni. The strain accumulated Ni ions inside the cell. The reduced Ni toxicity in high salt is attributed to the formation of the less toxic forms of the metal and/or the change in the membrane of the cell in such a way that causes higher level of metal tolerance.

Many *Halomonas* species have been reported to harbor plasmids of ~600 and ~70 Mbp, as well as other extrachromosomal elements. *H. elongata* and *H. subglaciescola* (Nieto et al., 1989) harbor ~600 and ~70 kbp plasmids (Argandona et al., 2003). These plasmids could be responsible for some of the adaptive advantages in the genus *Halomonas*, including tolerance to metals.

Amoozegar et al. (2005) isolated 10 moderately halophilic spore-forming bacilli from saline soils in Iran. All isolates were resistant to higher concentrations of arsenate, sodium chromate, and potassium chromate. Maximum and minimum tolerances against oxyanions were seen in selenite and biselenite, respectively. All isolates were susceptible to silver, nickel, zinc, and cobalt, while seven isolates were resistant to lead. Susceptibility to copper and cadmium varied among the isolates. Silver had the maximum toxicity, whereas lead and copper showed minimum toxicity. An increase in salinity from 5% (w/v) to 15% (w/v) enhanced tolerance to toxic oxyanions and metals. The only exception was cobalt, for which the maximum tolerance was seen in 5% (w/v) NaCl-containing medium. The maximum effect of the NaCl concentration on the toxic metal resistance was seen in the Pb-containing medium.

Massadeh et al. (2005) have reported isolation of 10 gram-positive and gram-negative bacterial cultures able to grow at 10% NaCl from water, mud, and soil samples from the shores of the Dead Sea in Suwaymah. Among them, maximum resistance to Pb was shown by two bacterial cultures in nutrient media, and seven bacterial cultures exhibited maximum resistance to Cd. However, the isolates were more resistant to lead than cadmium. The most tolerant gram-negative rod and gram-positive cocci were able to remove lead and cadmium from different concentrations with almost similar efficiency after 2–3 weeks. The accumulation of the absorbed metals was found to be maximum in the protoplast of all the cultures. The accumulation on the cell wall and between the cell wall and the plasma membrane varied for both the metals, and it differed according to strains also.

Bioluminescent bacteria *Vibrio harveyi* and TEMO5 and TEMS1 were very resistant to Pb and As and Pb, respectively, in nutrient medium with 2% NaCl. The strains are very sensitive to Cu, Cd, and Hg. MIC values of Mn, Ni, Zn, Pb, and Cr against *V. harveyi* TEMO5 were different from that of *V. harveyi* TEMS1. This indicated isolate-specific differences in the metal sensitivity (Omeroglu et al., 2007). Amoozegar et al. (2007) reported complete reduction of 0.2 m MCr(VI) after 24 h by halophilic *Nesterenkonia* sp. strain MF2 in the presence of 1 M NaCl.

Kim et al. (2007) in their study on a Pb-resistant *Bacillus* sp. strain CPB4 isolated from heavy metal–contaminated soil in Korea showed that 90% of Pb was distributed in the cell wall and cell membrane and the rest in the cytoplasm. In Pb uptake cells, electron-dense granules were mainly found on the cell wall and cell membrane. Its metal uptake capacity was in the following order: Pb > Cd > Cu > Ni > Co > Mn > Cr > Zn. In Cd uptake cells, electron-dense granules were mainly found on the cell membrane. However, in Cu-adsorbed cells, electron-dense granules were mainly found on the outside but located very close to the cells. These results show that the CPB4 cell has a variable pattern of biosorption with different heavy metals. The amount of heavy metal uptake was remarkably decreased by reducing the crude protein contents when cells were treated by alkali solutions (Kim et al., 2007).

De et al. (2008) reported that marine bacteria highly resistant to mercury were capable of detoxifying not only Hg but also Cd and Pb. These bacteria identified belong to the species *Alcaligenes faecalis*, *Bacillus pumilus*, *Bacillus* sp., *P. aeruginosa*, and *Brevibacterium iodinium*.

The mechanisms of heavy metal detoxification were through volatilization (for Hg), putative entrapment in the EPS (for Hg, Cd, and Pb) as revealed by the SEM and energy-dispersive x-ray spectroscopy (EDS), and/or precipitation as sulfide (for Pb). These bacteria removed Cd and Pb effectively from a growth medium.

Kawasaki et al. (2008) isolated three strains of genus *Staphylococcus* and one strain of genus *Halobacillus* with high salt resistance and cadmium-absorbing capability from salt-fermented food. Strains showed high removal of cadmium in a medium with salt concentrations of 0%–20%. The isolates could be used for reducing the cadmium in foodstuffs.

Ibrahim et al. (2011) isolated and characterized a novel potent Cr (VI)-reducing alkaliphilic *Amphibacillus* sp. KSUCr3 from hypersaline soda lakes. The strain tolerated very high Cr (VI) concentration in addition to high concentrations of other heavy metals including Pb, Ni, Mo, Co, Mn, Zn, and Cu. Strain KSUCr3 could rapidly reduce 5 mM of Cr (VI) to a nondetectable level over 24 h, and its reduction capacity increased with the increase in NaCl concentration. Khodabakhsh et al. (2011) reported a moderately halophilic bacterium grown in the Ventosa medium with 98% homology with *Salinivibrio costicola* species that is resistant to some toxic metals and has the potency of removing nickel from the contaminated environment.

Periplasmic metal-binding protein termed histidine-rich metal-binding protein (HP)—characterized by high histidine content—was reported from a moderate halophile *Chromohalobacter salexigens* (Yamaguchi et al., 2012). The study on the purified protein showed high affinity toward Ni- and Cu-loaded chelate columns and moderate affinity toward Co and Zn columns. Its structure was found to be stable at 0.2–2.0 M NaCl, and the thermal transition pattern was considerably shifted to higher temperature with increasing salt concentration. The melting temperature was raised to ~20°C at 2.0 M NaCl over the melting temperature at 0.2 M NaCl. HP showed reversible refolding from thermal melting in 0.2–1.15 M NaCl, while it formed irreversible aggregates upon thermal melting at 2 M NaCl.

Solanki and Kothari (2012) isolated *Virgibacillus salaries*, *Staphylococcus epidermidis, Bacillus atrophaeus, Halomonas shengliensis*, and *Halomonas koreensis* from saline soil of Gujarat. *V. salaries* exhibited better metal tolerance/resistance than other isolates. In certain cases, the stimulatory effect of metal ions on their growth was also observed. According to them, silver had the most toxic effect on all isolates followed by cadmium.

Amoozegar et al. (2012) isolated 24 moderately halophilic bacteria from saline environments of Iran that were used to study their ability to bioremediation of lead and cadmium. Among them, a *Halomonas* strain D showed remarkable ability for the removal of Pb and Cd. The strain D could uptake lead more effectively than cadmium. Biomass showed the best lead removal at 5% NaCl (w/v), while EPS showed maximum removal at 10% NaCl (w/v). For cadmium removal by biomass and EPS, the best results were obtained at 1% NaCl (w/v). In this study, the removal rate was much higher when using the biomass of strain D. The best metal removal results by biomass were obtained at NaCl concentrations near to optimum NaCl concentration for the growth of strain D. Whereas in the case of metal removal by exopolymeric substances, increasing NaCl concentration led to the best Pb removal rate and decreasing NaCl concentration led to the best Cd removal rate.

Multimetal-resistant bacteria belonging to alkaliphilic (*Natronobacterium magadii*, *Natronococcus occultus*, and *Nb. Gregory*) and halophilic (*Halobacterium saccharovorum*, *Hb. Sodomense*, and *Hb. salinarium*) groups were isolated from solar salt pan sediment at the coastal area of Tamil Nadu, India. They were resistant to Ni, Al, Cd, Zn, Hg, and As. The maximum percentage of resistance was identified for Ni, while the minimum percentage of tolerance was identified with Hg. None of the selected isolates showed the presence of plasmids. This indicated that the resistance of the chosen metals was directly controlled by the chromosomal DNA and the resistance activity of the halobacterial species might be related to metal-binding proteins (Williams et al., 2013).

Guo and Mahillon (2013) isolated pGIAK1—a 38 kb plasmid—originating from the obligate alkaliphilic and halotolerant *Bacillaceae* strain JMAK1. The strain was originally isolated from the confined environments of the Antarctic Concordia station. The analysis on the pGIAK1 38,362-bp sequence revealed that in addition to its replication region, this plasmid contains the genetic determinants for cadmium and arsenic resistances, putative methyl transferase, tyrosine recombinase, spore coat protein, and potassium transport protein, as well as several hypothetical proteins.

Halomonas lionensis RHS90T is a halotolerant bacterium highly sensitive to Ag and Cd but grew very well at high concentrations of Cs and was also resistant to Mn. The strain RHS90T contains one or several plasmids >10 kbp that might possibly be involved in metal tolerance (Gaboyer et al., 2014).

Halomonas zincidurans B6T capable of resisting high concentrations of heavy metals in liquid halophilic medium, including Mn, Co, Cu, and Zn, was reported from sediments of the South Atlantic Mid-Ocean Ridge. Its resistance to Zn was much higher when incubated on a marine agar 2216 medium. This *H. zincidurans* B6T was found to encode 31 genes related to heavy metal resistance (Huo et al., 2014).

The genome of *Lunatimonas lonarensis* AK24T shows the presence of heavy metal tolerance genes, including two genes providing resistance against arsenic and 11 genes for cobalt–zinc–cadmium resistance. This gram-negative, pinkish-orange-pigmented, half-moon-shaped bacterium was first isolated from water and sediment samples of Lonar Lake, Buldhana district, Maharashtra, India (Srinivas et al., 2014).

Vela-Cano et al. (2014) isolated eight strains from a sewage sludge compost tea with high resistance to Pb, Zn, Cu, and Cd and one with (*Rhodococcus* sp.) a special tolerance to every heavy metal tested. The strains belonged to *Rhodococcus*, *Virgibacillus*, *Leifsonia*, *Achromobacter*, *Cupriavidus*, and *Oceanobacillus* sp. *Rhodococcus* sp. strain 3 was able to remove different amounts of heavy metals from the culture media with intracellular and surface accumulation of Cu, Zn, Pb, and Cd. The tolerance of the tested strains was observed to be in the order of Cu > Pb > Zn > Cd. *Rhodococcus* sp. strain 3 showed maximum removal of lead (Pb) followed by Cu, Zn, and Cd. TEM analysis of *Rhodococcus* strain 3 grown in the presence of Cu, Cd, Zn, and Pb showed that Cu was mainly deposited on the cell surface structures, while small amounts of Cu were detected inside the cells. In the case of Cd, more metal could be detected inside the cells than outside. The analysis confirmed the bioaccumulation of Pb and Zn because both metals were mainly deposited inside the cells.

Moderately halophilic and halotolerant bacteria showing resistance to cadmium and lead were isolated by Sowmya et al. (2014) from the sediment samples of Vembanad Lake, India. Bacterial strains belonging to different genera such as *Alcaligenes, Vibrio, Kurthia,* and *Staphylococcus* and members of the family *Enterobacteriaceae* were reported. The isolates showed higher resistance to lead than cadmium at 5%, 10%, and 15% salt concentrations. Selected isolates removed lead more efficiently than cadmium.

Syed and Chinthala (2015) reported the detoxification potential of three *Bacillus* species isolated from solar salterns, namely, *B. licheniformis* NSPA5, *B. cereus* NSPA8, and *B. subtilis* NSPA13 against lead, chromium, and copper, by biosorption in metal biosorption medium (8.1% NaCl). *B. cereus* NSPA8 showed maximum lead biosorption. The biosorption of copper and chromium was relatively low in comparison with lead. Energy-dispersive x-ray system and EDS spectral images gave evidences of the binding of metal ions [Cd, Cu, and Pb ions] on the surface of the cell wall of the bacterial cells.

The works demonstrating the cadmium and lead removal efficiency of halotolerant/moderately halophilic bacteria in a saline medium or the effect of salinity variable on metal reduction are relatively less (Table 34.1).

Nonhalophilic halotolerant bacteria like *Pseudomonas* and *Staphylococcus* are extensively studied for their metal tolerance and tolerance mechanisms. However, even in those studies salinity was not included as a variable. A brief summary of those works is given in Table 34.2.

TABLE 34.1

Metal Resistance of Halotolerant/Moderately Halophilic Bacteria in Saline Medium

Sl. No.	Name of Bacteria	Medium/Salt Percentage (w/v)	Tolerant Heavy Metals	Resistance Mechanism(s)	References
1.	Bacteria and fungi	NB[a]	Zn	—	Babich and Stozky (1978)
2.	Moderately halophilic *Pseudomonas* sp.	SGC[b] 5.8%–17.5%	Cd		Onishi et al. (1984)
3.	*Vibrio costicola* (different strains)	BM[c] + 5% and 15% NaCl	Co < As < Cr < Ni < Pb (Cu, Hg, Ag, Cd, and Zn)[n]	—	Garcia et al. (1987)
4.	*Deleya*, *Acinetobacter*, *Flavobacterium,* gram-positive cocci	SW-10[d] broth	As, Cd, Cr, and Cu (Ag, Hg, and Zn)[n]	—	Nieto et al. (1989)
5.	Moderately halophilic *Staphylococcus* sp.	HM[e]	Ni (Cd and Cu)[n]	—	Gaballa et al. (2003)
6.	Moderately halophilic spore-forming bacilli	NB[a] + 5%, 10%, and 15%	Cd < Cu < Pb (Ag, Ni, Zn, and Co)[n]	—	Amoozegar et al. (2005)
7.	Moderately halophilic gram-negative rod and gram-positive cocci	SW-10[d] broth medium	Cd < Pb	Biosorption and bioaccumulation	Massadeh et al. (2005)
8.	*Halomonas maura* S-30		Pb	Binding on EPS—mauran	Arias et al. (2003); Llamas et al. (2006)
9.	*Halomonas ventosae* Al12T *H. ventosae* Al16, *Halomonas anticariensis* FP35T and FP36		Co < Pb Co < Cu	Binding on EPS	Mata et al. (2006)
10.	*Vibrio harveyi* TEMO5 and *Vibrio harveyi* TEMS1	NB[a] + 2%	As < Pb Pb = As (Cu, Cd, and Hg)[n]	—	Omeroglu et al. (2007)
11.	Halophilic *Nesterenkonia* sp. MF2	5.8%	Cr		Amoozegar et al. (2007)
12.	*Bacillus* sp. strain CPB4		Zn < Cr < Mn < Co < Ni < Cu < Cd < Pb	Electron-dense granules on cell surface and cytoplasm	Kim et al. (2007)
13.	*Alcaligenes faecalis*, *Bacillus pumilus*, *Bacillus* sp., *Pseudomonas aeruginosa* and *Brevibacterium iodinium*	SWN[f]	Hg Hg, Cd, and Pb Pb	Volatilization Binding on EPS Precipitation as sulfide	De et al. (2008)
14.	*Staphylococcus* sp. *Halobacillus* sp.	0%–20%	Cd	—	Kawasaki et al. (2008)
15.	Alkaliphilic *Amphibacillus* sp. KSUCr3	AM[g] + 0%–25%	Cu = Zn < Co < Cr(VI) = Mo = Pb < Ni = Mn	—	Ibrahim et al. (2011)
16.	*Salinivibrio costicola*	VM[h]	Ni, Cd, Co, Zn, Cu, Pb, Ag	—	Khodabakhsh et al. (2011)
17.	*Chromohalobacter salexigens*		Co and Zn < Ni and Cu	HP indicates Histidine-rich metal binding protein	Yamaguchi et al. (2012)
18.	*Virgibacillus salaries*, *Staphylococcus epidermidis, Bacillus atrophaeus, Halomonas shengliensis*, and *Halomonas koreensis*		Ag < Cd < Ni	—	Solanki and Kothari (2012)

(*Continued*)

TABLE 34.1 (*Continued*)
Metal Resistance of Halotolerant/Moderately Halophilic Bacteria in Saline Medium

Sl. No.	Name of Bacteria	Medium/Salt Percentage (w/v)	Tolerant Heavy Metals	Resistance Mechanism(s)	References
19.	*Halomonas* strain D	NA[i] + 10%; SM[j] + 5%	Cd < Pb	Biosorption, bioaccumulation, binding on EPS	Amoozegar et al. (2012)
20.	*Natronobacterium magadii, Natronococcus occultus,* and *Nb. Gregory*; *Halobacterium saccharovorum, Hb. sodomense,* and *Hb. salinarium*	HA[k]	Hg, Cd, As, Zn, Al, Ni	—	Williams et al. (2013)
21.	*Halomonas lionensis* RHS90T		Mn < Cs (Ag and Cd)[n]	Plasmid encoded	Gaboyer et al. (2014)
22.	*Halomonas zincidurans* strain B6T	HM[e]	Mn, Co, Cu, and Zn	Gene encoded	Huo et al. (2014)
23.	*Rhodococcus, Virgibacillus, Leifsonia, Achromobacter, Cupriavidus,* and *Oceanobacillus* sp.	LPM[l]	Cd < Zn < Pb < Cu	Intracellular and surface accumulation	Vela-Cano et al. (2014)
24.	*Alcaligenes, Vibrio, Kurthia, Staphylococcus,* and members of the family *Enterobacteriaceae*	NB[a] + 5%, 10%, and 15%	Cd < Pb	—	Sowmya et al. (2014)
25.	*Pseudomonas aeruginosa* JP-11	SWN[f] agar	Cd	Binding on EPS and gene encoded	Chakraborty and Das (2014)
26.	*Bacillus licheniformis* NSPA5, *Bacillus cereus* NSPA8, and *Bacillus subtilis* NSPA13	MBM[m]	Cr, Cu < Pb	Binding to cell wall	Syed and Chinthala (2015)

[a] Nutrient broth.
[b] Sehgal and Gibbons complex medium.
[c] Basal medium.
[d] Saline yeast extract medium.
[e] Halophilic medium.
[f] Seawater nutrient agar.
[g] Alkaline medium.
[h] Ventosa medium.
[i] Nutrient agar.
[j] Saline medium.
[k] Halophilic agar.
[l] LPM medium.
[m] Metal biosorption medium.
[n] Metals to which the isolates were sensitive.

34.5 CADMIUM AND LEAD RESISTANCE MECHANISMS

From the earlier discussions, it is clear that both cadmium and lead are externally sequestered on the EPS and cell wall of various bacteria. It is evident in SEM, TEM, and AAS analyses. In the biosynthesis of NPs, many bacteria were found to produce NPs externally. In most studies, we can see that lead tends to bind more on EPS/cell wall than cadmium. Though external and internal precipitation was reported for lead in some bacteria, only few works were reported on halotolerant bacteria. In the case of cadmium, intracellular sequestration and efflux mechanisms mediated by RND, CDF, and P-type ATPase transporters are more important. The main mechanisms of cadmium and lead tolerance are shown in Figures 34.2 and 34.3.

TABLE 34.2
Metal-Resistant Nonhalophilic Bacteria

Sl. No.	Bacteria	Metals Studied	Reference
1.	*Pseudomonas tolaasii* RP23 and *Pseudomonas fluorescens* RS9	Zn and Cd	Amico et al. (2005)
2.	*Pseudomonas aeruginosa* KUCd1	Cd	Sinha and Mukherjee (2008)
3.	Gram-positive bacilli	Fe, Pb, Hg, Zn, Al, Cu, Ni, Cd, and Ag	Roy et al. (2008)
4.	*E. coli* ASU7	Cu, Co, Zn, Ni, Pb, Cd, Cr^{6+}, and Cr^{3+}	Abskharon et al. (2008)
5.	*Pseudomonas aeruginosa, P. alcaligenes, Methylococcus* sp., and *Desulfotomaculum* sp.	Zn, Cu, Ni, Cd, and Cr	Mishra et al. (2009)
6.	*Proteus vulgaris* (BC1), *Pseudomonas aeruginosa* (BC2 and BC5), and *Acinetobacter radioresistens* (BC3)	As, Ni, Cd, Pb, Hg, and Cr	Raja et al. (2009)
7.	*Pseudomonas aeruginosa* KUCd1	Cd	Sinha and Mukherjee (2009)
8.	Purple nonsulfur bacteria (PNB) NW16 and KMS24	Pb, Cu, Cd, Zn, and Na	Panwichian et al. (2010)
9.	*Pseudomonas aeruginosa* MCCB 102	Cu, Zn, Cd, and Pb	Zolgharnein et al. (2010)
10.	*Aeromonas* sp. and *Pseudomonas* sp.	Cu, Pb	Matyar et al. (2010)
11.	*Bacillus cereus* SIU1	As, Pb, and Cs	Singh et al. (2010)
12.	*Pseudomonas aeruginosa*	Cd	Kermani et al. (2010)
13.	*Pseudomonas* sp.	Pb	Dinu et al. (2011)
14.	*Pseudomonas putida*	Cd	Nanganuru and Korrapati (2012)
15.	*Pseudomonas, Aeromonas, Bacillus, Escherichia, Micrococcus,* and *Proteus species*	Fe, Zn, and Pb	Mgbemena et al. (2012)
16.	*Bacillus* sp., *Pseudomonas* sp., *Corynebacterium* sp., *Staphylococcus* sp., and *E. coli*	Pb	Kafilzadeh et al. (2012)
17.	*Pseudomonas* sp., *Klebsiella* sp., *Staphylococcus* sp., *Proteus* sp., and *Bacillus* sp.	Cd and Pb	Nath et al. (2012)
18.	*Bacillus cereus*	Pb	Murthy et al. (2012)
19.	*Actinomyces* sp., *Streptomyces* sp., and *Bacillus* sp.	Cd and Ni	Karakagh et al. (2012)
20.	*Bacillus* sp.	Cd, Cr, Ni, and Co	Samanta et al. (2012)
21.	*Enterobacter cloacae* P2B	Pb	Naik et al. (2012)
22.	*Massilia* sp., *Pseudomonas* sp., *Pseudomonas fulva, Bacillus* sp., and *Serratia entomophila*	Cd	Złoch et al. (2013)
23.	*Bacillus cereus, Bacillus subtilis* subsp. *subtilis*	Hg, Pb, Ag, Zn, and Cu	Hookoom and Puchooa (2013)
24.	*Pseudomonas putida* and *Bacillus licheniformis*	Mn, Cu, Ni, Co, V, Pb, Ti, Al, and Zn	Kamika and Momba (2013)
25.	*Staphylococcus aureus*	Cd	Shrivastava et al. (2013)
26.	Cadmium-resistant bacterium CdRB1	Cd	Dabhi et al. (2013)
27.	*Bacillus cereus*	Pb	Murthy et al. (2014)
28.	*Stenotrophomonas maltophilia, Bacillus halodurans, Exiguobacterium homiense, Pseudomonas putida,* and *Pseudomonas geniculata*	Cd, Cr, Zn, Mg, Co, and Ni	Prabhu et al. (2014)
29.	*Bacillus, Lysinibacillus, Micrococcus, Stenotrophomonas, Bacillus pumilus* Jo2, *Bacillus subtilis* BD18−B23, and *Lysinibacillus fusiformis* B	Cr, Cu, Ni, Co, Cd, Zn, and Pb	Tomova et al. (2014)
30.	*Aeromonas* sp., *Arthrobacter* sp. *Corynebacterium* sp., *Pseudomonas* sp., *Streptococcus* sp.	Pb, Cr, and Cd	Owolabi and Hekeu (2014)
31.	*Bacillus subtilis* KPA	Ag, Hg, Cu, and Cr	Khusro at al. (2014)
32.	*Pseudomonas* sp. PMDZnCd2003, *Serratia* sp. PDMCd2007, and *Serratia* sp. PDMCd0501	Cd and Zn	Nakbanpote et al. (2014)
33.	*Klebsiella pneumoniae, Pseudomonas aeruginosa, Providencia rettgeri, Alcaligenes faecalis, Morganella morganii,* and *Pseudomonas putida*	Zn, Hg, and Cd	Yamina et al. (2014)
34.	*Massilia* sp., *Pseudomonas* sp., and *Bacillus* sp.	Cd	Hrynkiewicz et al. (2015)
35.	*Bacillus* sp., *Enterobacter* sp., *Aeromonas* sp., and *Pseudomonas* sp.	Cd	Mathivanan and Rajaram (2014)
36.	*Staphylococcus epidermidis* ATCC 35984	Cd	Wu et al. (2015)
37.	*Klebsiella* sp. 3S1	Pb	Munoz et al. (2015)
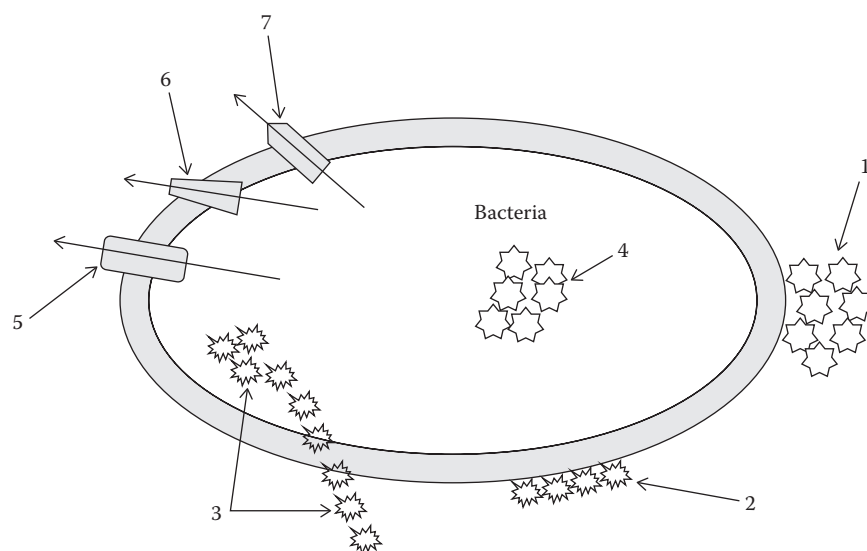
FIGURE 34.2 Cadmium-resistant mechanisms in bacteria. 1, extracellular sequestration on EPS; 2, CdS NPs binding to cell surface; 3, CdS NPs formed intracellulary and coming outside through cell decay; 4, intracellular sequestration/precipitation; 5, efflux mechanisms by RND (Czc, Ncc); 6, efflux mechanisms by CDF (ZntA); 7, efflux mechanisms by P-type ATPase (CadA). (Modified from Jarosławiecka, A. and Piotrowska-Seget, A., *Microbiology* (*United Kingdom*), 160, 12, 2014.)
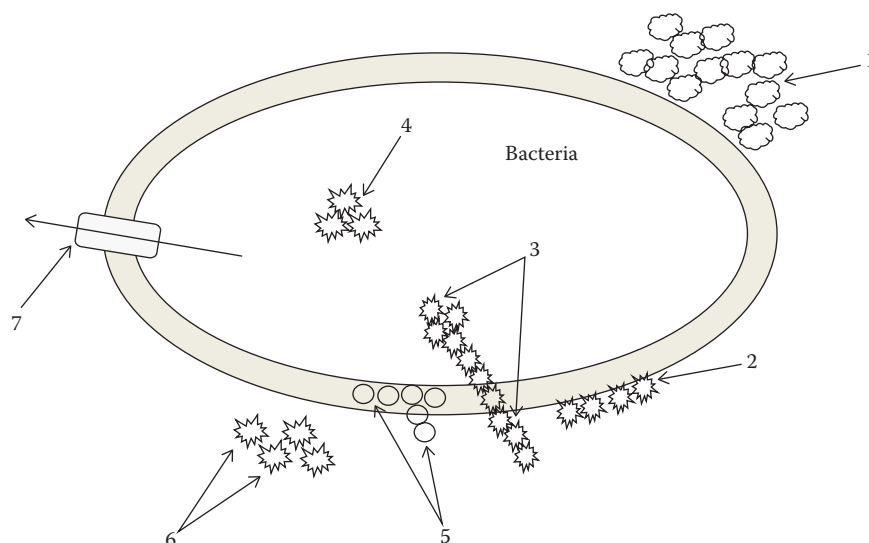
FIGURE 34.3 Lead resistance mechanisms in bacteria. 1, extracellular sequestration on EPS; 2, PbS NPs binding to cell surface; 3, PbS NPs formed intracellulary and coming outside through cell decay; 4, intracellular sequestration/precipitation; 5, periplasmic PbO NPs coming outside through cell decay; 6, extracellular precipitation; 7, efflux mechanisms like PbrA, CadA, and ZntA. (Modified from Naik, M.M. and Dubey, S.K., *Ecotoxicol. Environ. Saf.*, 98, 1, 2013; Jarosławiecka, A. and Piotrowska-Seget, A., *Microbiology* (*United Kingdom*), 160, 12, 2014.)

34.6 SHORTCOMINGS AND FUTURE ASPECTS OF HALOTOLERANT AND MODERATELY HALOPHILIC BACTERIA IN CADMIUM AND LEAD BIOREMEDIATION

In this chapter, the general mechanisms of heavy metal resistance in bacteria and the potential of halophilic bacteria in bioremediation with special reference to cadmium and lead have been reviewed. In the present scenario, we need strains with high metal resistance and removal capacity in varying salinities. It is a well-known fact that the constituents of bacterial growth media and also the salt will react with cadmium and lead. The toxicity of a metal to the bacteria strongly depends on its bioavailability rather than its concentration. At higher concentrations, lead tends to precipitate more in the media. So when conducting metal reduction experiments, care should

be given to the constituents of media and the concentration of the metal tested. Though there were a number of studies dealing with the interference of culture medium components and salinity with metals, there is still a long way to go.

In the case of biosurfactants and NPs, studies on halotolerant bacteria are less compared with other microorganisms. The efficiency of these moderately halophilic and halotolerant microbes has to be exploited in saline conditions for the production of Cd/Pb-binding biosurfactants and Cd/Pb NPs. These organisms have to be more elaborately studied for their metal removal efficiencies.

Biomineralization also presents a prospective area to be studied in detail. It is a very important natural process. So the influence of Cd/Pb on the process and the ability of bacteria to remove them from the environment in the due course have to be explored.

Different aspects have to be studied together. With multi-metal resistances, other capabilities of the microbe like COD removal have to be addressed. Emphasis has to be given on the recovery of metals from saline effluents.

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