Chapter 15
Metallotolerant Microorganisms and Microbe-Assisted Phytoremediation for a Sustainable Clean Environment

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Abstract Both natural and anthropogenic activities have upsurged the accumulation of heavy metals in the environment. These pollutants affect the natural ecosystems, and on entering the food chain, they become hazardous to public health. In the polluted soil, where survival of plants and microbes is difficult, metallotolerant microbes can thrive by tolerating the toxic effects of heavy metals. For that, they use diverse survival mechanisms which also assist them to perform bioremediation. In comparison to conventional and physical methods of conversion of the toxic effect of metals to its non-toxic form, bioremediation is a more effective method for retrieving the metal-contaminated environments and convert the degraded area into green covers. Considering the importance, this book chapter sheds light on the mechanism, which encourages the metallotolerant microbes thriving in these metal-rich environments and performs bioremediation.

Keywords Soil · Heavy metals · Metallotolerant microbe · Bioremediation · Microbe-assisted phytoremediation

15.1 Introduction

Land degradation is among the most imperative problems facing the world today. Approximately, one-third of the earth's land surface is degraded, affecting more than 2.6 billion people. Degradation of land is mainly caused by the accumulation of elevated level of heavy metals released due to various geological and anthropogenic activities including mining, industrial emissions, fertilizer erosion from agricultural run-off, sewage, and municipal wastes (Sharma and Nagpal 2020; Romaniuk et al. 2018). It is estimated that heavy metals or metalloids have affected approximately five million sites around the globe (Liu et al. 2018). Various reports are claiming that

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the high content of heavy metals converts fertile land to degraded one in many parts of the world (Sharma and Nagpal 2020). In India, approximately 55% of the geographical area is degraded, and out of which, mining activities have degraded approximately 0.8 mha (MOEF 2001). Most of these heavy metals are generally nondegradable, and the persistent nature of these heavy metals for a longer period in aquatic and terrestrial ecosystems consequently creates harsh conditions for plant growth and development. This is responsible for the conversion of the green landscape of an area into degraded land (Sarma and Barik 2011). Among various heavy metals, Co, Cu, Fe, Mn, Mo, Ni, V, and Zn are required in minute quantities by organisms, but it becomes harmful to organisms with their presence in excessive amounts. There are some other heavy metals like Pb, Cd, Hg, and As which do not have any beneficial effect and regarded as the major threats to organisms (Chibuike and Obiora 2014; Singh et al. 2020a; Barman et al. 2020). The United States Agency for Toxic Substances and Disease Registry (ATSDR) has also listed As, Pb, Hg, and Cd as the major threat to human health (Wood et al. 2016). These heavy metals reduce plant growth by reducing photosynthetic activities, essential enzyme activities, and mineral nutrition (Ojuederie and Babalola 2017; Sivakumar 2016). This issue has attracted worldwide attention as heavy metals enter the food chain and cause detrimental impacts on human health. Heavy metals also enhance the production of reactive oxygen species (ROS) which causes a harmful effect on cells (Ojuederie and Babalola 2017).

Hence, it is imperative to remediate metal-contaminated soil. The treatment of soil using conventional methods including chemical precipitation, electrochemical treatment, and ion exchange is extremely expensive and adversely affects biological activity, soil structure, and fertility (Gupta et al. 2016). In contrast to conventional methods, bioremediation is increasingly gaining importance due to its low cost, simplicity, and better efficiency (Wei et al. 2014; Singh et al. 2017). Bioremediation was first commercially used to clean up the Sun Oil pipeline spill in Ambler, Pennsylvania during 1972 (National Research Council 1993). Since then, bioremediation has received increasing recognition for remediation of a contaminated site like Exxon Valdez and Mega Borg oil spills, Alaskan Oil Spills, and the Iraq–Kuwait war and its consequences (Shannon and Unterman 1993; Pritchard and Costa 1991). The Environmental Protection Agency in 1992 reported 240 cases of bioremediation in the United States (Alexander 1999). Despite the overwhelming advantages, the exact mechanisms by which microbes exist in such a type of environment and decontaminant pollutants are not precisely known.

Under metal stress conditions, some of the soil microorganisms (metallotolerant microorganisms) have developed certain mechanisms to avoid the toxicity arising due to the presence of an array of heavy metals. These mechanisms include an extracellular barrier, efflux of toxic ions from cells, incorporation of heavy metals into complexes by metal-binding proteins, enzymatic transformations of metals, bioaccumulation of the metal ions inside the cell actively or passively, etc. (Romaniuk et al. 2018). They can survive and detoxify heavy metals in polluted soil by expressing different metal-resistant genes (Crupper et al. 1999; Borremans et al. 2001; Yang et al. 2019). Microbes also facilitate bioremediation on interacting

with plants termed as microbe-assisted phytoremediation where microbes enhance the process of phytoremediation, as well as increase the growth and biomass of the hyperaccumulating plant at the polluted sites (Tirry et al. 2018). Microbes facilitate the bioavailability of heavy metals to plant by acidification, releasing chelating substances, and changing the redox potentials (Whiting et al. 2001). Besides, microbes facilitate plant growth in heavy metal-contaminated soils by phosphorus solubilization and N_2 fixation and by producing siderophores, phytohormones, antibiotics, and antifungal metabolites. They can also alleviate the ethylene-mediated stress on synthesizing 1-aminocyclopropane-1-carboxylate (ACC) deaminase which can improve plant stress tolerance to metals (Ahemad 2019). Therefore, these beneficial microbial strains can be used as biofertilizers that significantly enhance phytoremediation as well as the growth of plants in heavy metal-contaminated soils (Ahemad 2019).

Further, there are different environmental factors that greatly influence the process of bioremediation, i.e., concentration of contaminants, availability of nutrients, characteristics of soil of the contaminated site. Studies have implied that these factors control the efficiency of bioremediation by various mechanisms. Recently, the importance of genetically engineered microorganisms (GEMs) to remediate contaminated site has increased due to their efficient genetic makeup. But still, the application of GEMs in metal-contaminated site has been limited to laboratory trial only because of regulatory risk and ecological concerns. They also hamper the indigenous population of microbes due to their uncontrolled propagation and horizontal gene transfer. Hence, it is essential to construct the life cycle of GEMs and allowing their death as soon as the pollution level is decreased to minimize their detrimental effects on the native population.

Considering the global significance of bioremediation of heavy metal contaminated sites, it is necessary to critically analyze the various strategies adopted by microbes to survive in metal-contaminated environments and the speculative mechanisms underlying detoxification and/or removal of toxicity from the contaminated site. Additionally, the role of omics and multi-omics approaches in bioremediation also needs to be delineated. Moreover, we also analyzed different relevant published data on the contribution of microbes to remediate the heavy metal contaminated environments.

15.2 Effects of Heavy Metals

Heavy metals are ions with partially or filled *d*-orbital having an atomic weight ranging between 63.5 u and 200.6 u, specific gravity of greater than 5. The physicochemical properties like pH, organic matter, clay contents, inorganic anions, and cations of soil get changed due to the presence of heavy metals (Sarma and Barik 2011; Lauwerys et al. 2007). The toxic effects of heavy metals also change the population size, diversity, and activities of soil microbiota, which in due course affect the soil enzymatic activities, recycling of plant nutrients, and ultimately

hamper plant growth (Karaca et al. 2010; Wang et al. 2007). It is interesting to note that plants growing in metal contaminated soils show abnormalities in their biochemistry and physiology (Chibuike and Obiora 2014). For example, the presence of arsenic (As) in the soil leads to decreasing seed germination, reduction of seedling height, leaf area, and declining production of dry matter in *Oryza sativa*. Arsenic (As) also causes chlorosis, wilting, and stunted growth in *Brassica napus* while it inhibits the rate of transpiration of *Avena sativa* seedlings. Similarly, the presence of Pb in soil results in stunted growth, reduced germination percentage, protein content, and biomass of *Zea mays*, and inhibited ribulose-1,5-bisphosphate carboxylase/oxygenase activity that affected CO_2 fixation in *Avena sativa* (Chibuike and Obiora 2014). These effects are attributed to the inhibition of vital metabolic processes of plants like photosynthesis, water absorption, mitosis that sometimes lead to the death of the affected plants (Shun-hong et al. 2009). It is worth mentioning that, due to mining activities, generally soil become polluted not only with one heavy metal but with a combination of heavy metals which results in more harmful effects to plants (Chibuike and Obiora 2014). It was observed that the combination of Pb and Cu at high (1000 mg/kg each) and low concentrations (500 mg/kg) in soil cause fast death of the leaves and stems of *Lythrum salicaria* (Nicholls and Mal 2003). The uptake of heavy metals by plants and its consequent accumulation along the food chain also caused depletion of essential nutrients in the body that further resulted in cancer in humans, decreasing immunological defenses, intrauterine growth retardation, and disabilities associated with malnutrition (Ojuederie and Babalola 2017).

15.3 Bioremediation

Bioremediation is the eco-friendly, efficient technique to remove heavy metals from the contaminated site (Dixit et al. 2015). Bioremediation is of two main types, i.e., in situ or ex situ. In situ bioremediation involves a process where the indigenous microorganisms are stimulated to degrade heavy metals on supplying nutrients and oxygen with negligible or not interfering the soil structure. This technique has been successfully used to treat metal-contaminated site and is found to be less expensive and superior than ex situ bioremediation (Roy et al. 2015).

The in situ bioremediation process can be enhanced by chemotaxis, and the formation of biosurfactants or biofilm. Chemotaxis is a phenomenon that guides microbes to move toward or away in response to a chemical stimulus which helps in decontamination of pollutants (Ahmad et al. 2020). This behavior is not only useful for nutritional requirements but also required for their interaction with the environment. Microbes generally move toward a chemical when they utilize it for their growth and move away from a chemical when it is toxic. Microbes also form biofilm or biosurfactants to survive in metal-contaminated environments and thus enhance their bioremediation potential. It has been reported that *Pseudomonas* sp. produces biofilm to tolerate the toxicity of cadmium ion, and *Rhodotorula mucilaginosa* produces biofilm to remove toxicity of heavy metals (Tarekegn et al. 2020; Chien

et al. 2013). In situ bioremediation can also be enhanced by improving native microorganisms by genetic engineering.

Ex situ bioremediation involves the transfer of contaminated pollutants from the original site to a different location for the treatment depending on the type of pollutants, cost of treatment, degree of pollution, and geology of the polluted site (Ojuederie and Babalola 2017). Based on the physical condition of the pollutant, ex situ bioremediation is of two types, i.e., solid-phase bioremediation and semi-solid-phase bioremediation. Solid-phase bioremediation includes biopile, landfarming, and composting. Landfarming is the technique where contaminated soil is excavated from the site and transported to a prepared bed to allow aerobic degradation by autochthonous microbes. Sometimes instead of transferring contaminated soil, they are treated on that site; hence, landfarming is also regarded an in situ bioremediation technique. In composting, excavated soil is mixed with compost to allow effective growth of native isolates and to permit bioremediation of contaminated soil. Bioremediation by biopile includes piling of contaminated soil and subsequently maintaining favorable condition for native microorganisms (Pande et al. 2020). Semi-solid-phase bioremediation is performed in a sludge bioreactor where polluted soil is mixed with liquid that favors better interaction between native microorganisms and pollutants (da Silva et al. 2020).

The efficiency of bioremediation depends upon several biotic and abiotic factors (Brar et al. 2006). The microorganism capable of performing degradation is affected by the characteristics of contaminants, chemical condition of the surrounding environment, and the other indigenous microflora and fauna. The competition between degrading microorganisms with other indigenous microflora and fauna for carbon sources leads to deficient conditions of nutrients, oxygen, and ultimately hamper their growth and to perform bioremediation successfully. The condition can be overcome by the application of bioaugmentation, repeated inoculation, and pre-induction (Pande et al. 2020). Bioremediation is also affected by various abiotic factors of the contaminated site. One of the most important factors is pH which has a high impact on biological activity (Singh et al. 2016a, b). Generally, bioremediation rate increased in the pH range 6.5–8.5, and it is hampered above and below this. Another important factor is temperature, and 30–40 °C is optimum for biodegradation. It has been observed that degradation of the contaminant is affected by very low or very high temperatures. The water-holding capacity of soil also affects the bioremediation process. Water is essential for the transportation of nutrients into microbes, oxygen exchange, and ejection of metabolic waste which directly influence its cell growth and efficiency to perform bioremediation. However, an excessive amount of water in soil prevents oxygen exchange and thus hamper bioremediation. Moreover, an adequate amount of nutrients are required for the growth of cells and their efficiency of biodegradation. Generally, metal-contaminated site deficiency of nutrients hampers the process of biodegradation, and it can be overcome by adding the nutrients in their useable form (Pande et al. 2020).

Though bioremediation has advantages over conventional techniques like less expensive method, it can be done on site, can permanently eliminate waste, and has

more public acceptance (Boopathy 2000); however, the process of bioremediation is linked with some limitations like site-specificity where bioremediation approaches that are successful at one site may not be fruitful in other sites. Second, the microbe-mediated bioremediation process may fail in the field even it is successful under lab condition. Third, the uncertain mechanism of microorganisms is inhabiting in contaminated environments (Malla et al. 2018). Therefore, it is important to gather knowledge on the strategy used by microorganisms to grow in contaminated environments and subsequently perform bioremediation.

15.3.1 Microbial Strategies to Strive in Metal-Contaminated Environment and Underlying Mechanism

Most of the heavy metals disrupt the cell membrane of microorganisms, but the one capable of bioremediation is generally adapted to a range of resistance mechanisms through which they can utilize various toxic compounds as a source of energy for their growth and development and/or convert them into nontoxic products (Wei et al. 2014; Brar et al. 2006). Metallotolerant microbes tolerate the toxicity of heavy metals and perform bioremediation by different mechanisms like exclusion by permeability barrier, effluxing metal ions, oxidizing metals, enzymatic conversion of metals, intracellular and extracellular metal sequestration, producing metal chelators like metallothioneins and biosurfactants (Igiri et al. 2018).

Microbes can block the entry of heavy metals into the cell by using their extracellular membrane, i.e., plasma membrane, cell wall, and capsule. The extracellular surfaces are negatively charged which adsorb the positively charged heavy metals onto the binding sites of the cell wall by electrostatic interaction, ion exchange, precipitation, redox process, and surface complexation (Ayangbenro and Babalola 2017; Diep et al. 2018) (Fig. 15.1). On binding the heavy metals with the cell surface, microbes reduce their toxicity by transforming them from one oxidation state to another and thus prohibit the transportation of metal ions into the cytoplasm (Ayangbenro and Babalola 2017; Singh et al. 2020b). The phenomenon of uptake of heavy metals through surface complexation to the extracellular surface of microorganisms is termed as biosorption (Diep et al. 2018). The capacity of biosorption is influenced by three factors: (1) characteristics of the metal ion like an ionic ray, atomic weight, valence; (2) conditions of the environment such as pH, temperature, ionic strength, contact time, biomass concentration; and (3) the nature of the biosorbent (Perpetuo et al. 2011). The method of biosorption is of two types, i.e., metabolism-independent biosorption and metabolism-dependent biosorption. Metabolism-dependent biosorption mainly takes place within viable cells where metabolism occurs. Here metals get transported across the cell membrane and yield intracellular accumulation. However, metabolism-independent biosorption is mainly occurring on the exterior of cells and is a relatively rapid and reversible process (Perpetuo et al. 2011). If heavy metals enter into cytoplasm of the cell,

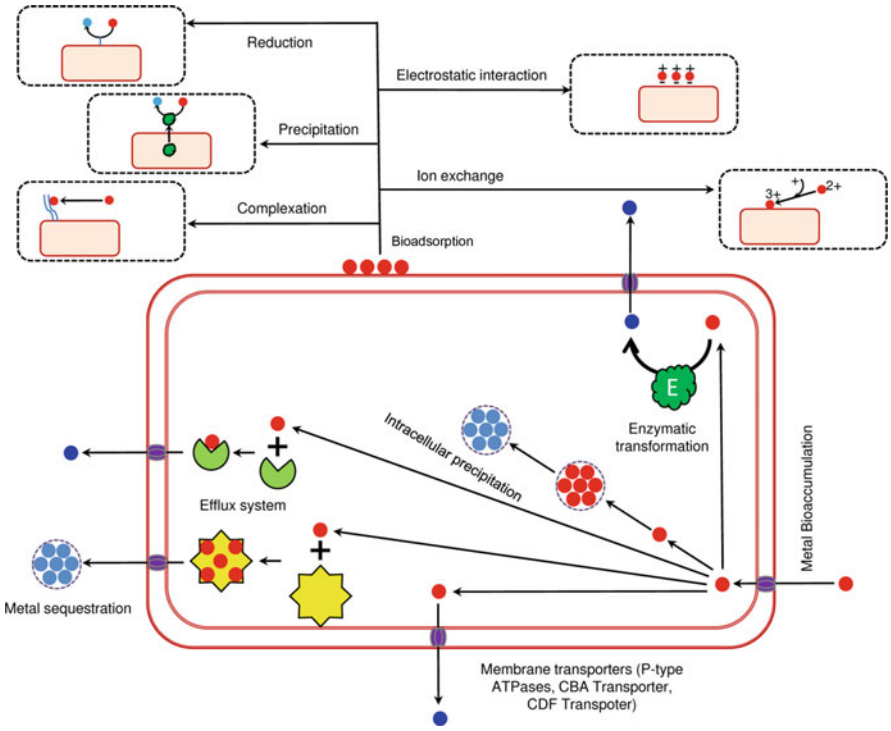

Fig. 15.1 A generalized illustration of different mechanisms involved in tolerance to toxic metals in bacteria

metallotolerant microbes efflux metal ions from the cytoplasm using three different proteins, i.e., resistance nodulation-cell division (RND superfamily) proteins, cation diffusion facilitators (CDF family), and P-type ATPases (Nies 2003).

Biotransformation is another mechanism by which microbes can detoxify the toxic effects of heavy metals. It includes oxidation, reduction, methylation, and alkylation or by synthesizing and producing metal-binding proteins such as metallothioneins (MTs) (Valls and de Lorenzo 2002) (Fig. 15.1). For example, *Alcaligenes faecalis* becomes resistant to toxic effects of arsenite [As(III)] on oxidizing arsenite to arsenate [As(V)] (Valls and de Lorenzo 2002).

$$\frac{1}{2}\,H_3AsO_3 + \frac{1}{4}\,O_2 \rightarrow \frac{1}{4}H_2AsO_4^- + \frac{1}{4}HAsO_4^{2-} + \frac{3}{4}\,H^+$$

Iron oxidizing bacteria reduce Fe(III) to Fe(II) abiotically; mercury (Hg^{2+}) into less toxic and volatile mercury (Hg°) by mercury reductase enzyme (Lloyd 2003; Valls and de Lorenzo 2002).

$$Hg^{2+} + NADPH \xrightarrow{\text{mercury reductase}} Hg^0 + H^+ + NADP^+$$

$$Fe_2O_3 + 3C \rightarrow 2FeO + 3CO$$

Some bacteria such as Clostridia, Methanogens, and Sulfate-reducing bacteria methylate a range of metals including lead, cadmium, tin, arsenic, selenium, tellurium, and mercury; as a result, the metals get transformed into their volatile dimethyl form (Igiri et al. 2018). In the process of alkylation, an alkyl group other than methyl group is directly bonded to metals through a carbon atom, for example, $As(C_2H_5)(CH_3)_2$, $As(C_2H_5)_3$, $As(C_2H_5)_2(CH_3)$, $Sb(C_2H_5)_3$ and by which it can tolerate the toxic effects of metals (Krupp et al. 1996). Microbes can also remove the toxicity of metals by synthesizing metallothioneins (MTs). For example, *Rhizobium leguminosarum* becomes cadmium resistant by sequestering cadmium ions by glutathione (Lima et al. 2006). Similarly, *Pseudomonas aeruginosa* strain WI-1 having metallothionein (BmtA) tolerates the toxic effect of lead by intracellular sequestration (Naik et al. 2012).

Microbes also precipitate lethal metal compounds intracellularly and/or extracellularly and thus convert them to less toxic form (Igiri et al. 2018). Metal precipitation is mainly achieved by dissimilatory metal reduction, sulfide precipitation, and phosphate precipitation (Valls and de Lorenzo 2002). In dissimilatory metal reduction, microbes precipitate metals such as uranium, selenium, chromium, technetium, and gold which is unrelated to its intake by microbial catalyst (Valls and de Lorenzo 2002). In sulfide precipitation, sulfur-reducing bacteria (SRB) precipitate metal [U (VI), Cr(VI), Tc(VI), Pd(II), and As(V)] in the form of metal sulfide on producing hydrogen sulfide (Igiri et al. 2018).

$$U(VI) + H_2S \rightarrow US_2$$

Similarly, some of the bacteria including *Vibrio harveyi, Citrobacter* sp. precipitate metal ions by producing highly insoluble metal phosphates (Valls and de Lorenzo 2002).

$$U(VI) + H_3PO_4 \rightarrow UP_2O_8$$

$$As(V) + H_3PO_4 \rightarrow As_3(PO_4)_5$$

Additionally, microbes like *Ralstonia metallidurans*, *Pseudomonas aeruginosa,* and *Alcaligenes eutrophus* detoxify toxic metals by forming metal-siderophore complexes. Siderophores are low-molecular-weight chelating agents having a strong affinity for ferric iron and thus produce Fe(III)-siderophore complexes. They also possess an affinity for other non-iron metals, e.g., copper, manganese, molybdenum, vanadium, zinc, which stimulate microbes to produce zincophores, chalkophores (copper-binding metallophores), etc. that can detoxify heavy metals. Microbes including various bacteria and yeast produce biosurfactants like rhamnolipids, lipopolysaccharides, exocellular polymeric surfactants in the form of

polysaccharides, proteins, lipoproteins by which they can solubilize and precipitate different heavy metals such as Cd, Pb, and Zn (Mosa et al. 2016; Valls and de Lorenzo 2002).

Microbes survive in a metal-contaminated niche by expressing metal-resistant genes generally associated with plasmids (Dave et al. 2020). There are certain operons, i.e., *cad* operon, *czc* operon, *ncc* operon, *mer* operon, *cop* operon, *aox*/*ars* operon present in the plasmid of microbes by which they can tolerate the toxicity of Cd, Zn, Ni, Hg, Cu, and As metals, respectively (Dave et al. 2020). The *cad* operon and *czc* operon are generally found in *Staphylococcus* sp. and *Pseudomonas aeruginosa,* respectively, by which the bacteria confer Cd resistance (Das et al. 2016). In a study, it was shown that *Ralstonia metallidurans* ch34 can resist Cu, Co, and Zn by *czc* operon (Dave et al. 2020). Similarly, *chrA* gene can encode the chromate reductase protein present in *Arthrobacter aurescens*, *Bacillus atrophaeus*, *Pseudomonas putida*, *Rhodococcus erythropolis* by which they can transform toxic Cr(VI) to the non-toxic Cr(III) with co-factors NADH or NADPH (Das et al. 2016). Lead is another toxic metal, and the *pbr* operon (lead resistance operon) found in the endogenous pMOL30 megaplasmid confers resistance to lead. The operon consists of one regulatory gene (*pbrR*), and many structural genes *pbrT*, *pbrA*, *pbrB*, *pbrC*, *pbrD* help microbes to resist lead. In the presence of lead toxicity, transcription of *pbrABCD* operon from *pbrA* promoter is induced which is regulated by *pbrR* (Borremans et al. 2001). Interestingly another gene *pbrU* was discovered in *Ralstonia metallidurans* by Monchy et al. (2007), which gets induced in the presence of lead. Microbes can also resist the toxicity of mercury by expressing two different operons, i.e., narrow-spectrum *mer* operon and the broad-spectrum *mer* operon (Silver and Phung 2013). The narrow-spectrum *mer* operon found on the transposons Tn5037 consists of the genes *merR*, *merT*, *merC*, *merF*, *merP*, and *merD*. The operon gets induced in the presence of Hg^{2+} that provides resistance to the metal. Similarly, the broad-spectrum *mer* operon contains the genes *merE*, *merG*, and *merB* in addition to the genes present in narrow-spectrum *mer* operon that protect from organic mercury (Barkay et al. 2003).

Microbes also occupy and adapt themselves in contaminated niche by horizontal gene transfer (HGT). The genes encoding bioremediation transfer through the action of conjugative plasmids, transposable elements, and "integrative and conjugative transposons." An interesting example of horizontal gene transfer is that the *pheBA* operon encodes enzymes involved in phenol catabolism which are originated from the *Pseudomonas* sp. EST1001. The operon was transferred to *P. putida* PaW85 by conjugation and released into river water contaminated with phenolics, originating from a fire in an oil shale mine for bioremediation. After 6 years, though the *P. putida* PaW85 was absent in that river water nonetheless the operon was detected in nine *Pseudomonas* strains in the watershed (Perpetuo et al. 2011). Another conjugative plasmid, i.e., IncP-specific plasmid sequences that are present in heavy metal contaminated soil gets mobilized to bacteria and offers resistance capacity of bacteria to survive in that environment by HGT (Ansari et al. 2008). Smalla et al. (2006) detected the abundance of IncP-1β plasmids and mercury-resistance genes in

mercury-polluted river sediments which were further detected in bacterial communities of that area indicating the role of HGT of IncP-1β plasmid.

15.3.2 Diversity of Metallotolerant Microorganisms

Several metal-tolerant microorganisms including bacteria, fungi, and algae have been used to remediate heavy metal–contaminated environments. Among the microorganisms, bacteria belonging to *Firmicutes*, *Proteobacteria*, and *Actinobacteria* play an important role in bioremediation due to their size, ubiquity, and ability to grow under controlled conditions as well as to their flexibility to varied environmental conditions (Igiri et al. 2018). They not only detoxify heavy metals in contaminated soils but also promote the growth and development of plants (Mishra et al. 2017). For the past few years, several articles have been published based on the use of bacteria for bioremediation purposes. Alboghobeish et al. (2014) isolated nickel-resistant bacteria from industrial waste waters belonging to *Cupriavidus* sp. ATHA3, *Klebsiella oxytoca* ATHA6, and *Methylobacterium* sp. ATHA7 which were found to remediate the Ni-polluted waste water and sewage. Bacteria can also successfully survive in mixed culture; hence, consortia of cultures can also be used for biosorption of metals and are found to more appropriate for field application (Igiri et al. 2018) (Table 15.1).

Fungi are also used as biosorbents for the removal of heavy metals. Both active and dead fungal cells play an important role in the adhesion of inorganic chemicals. Active fungal cells of *Saccharomyces cerevisiae*, *Aspergillus parasitica,* and *Cephalosporium aphidicola* were reported to detoxify Zn(II), Cd(II), and Pb (II) (Ayangbenro and Babalola 2017). White-rot fungi like *Phanerochaete chrysosporium*, *Trametes versicolor*, *Bjerkandera adjusta,* and *Pleurotus* sp. also transform a variety of organic pollutants by various ligninolytic enzymes. Marine fungi use enzymes to tolerate high concentrations of heavy metals like Pb and Cu (Deshmukh et al. 2016). The dead fungal biomass can also detoxify the toxic effect of metals. For that, the non-living biomass of *Rhizopus oryzae* and *Saccharomyces cerevisiae* use adsorption mechanism to convert toxic Cr(VI) to less toxic or non-toxic Cr(III) where anionic chromate ion binds to the cationic amines of the cell wall. However, the dead biomass of *Aspergillus niger* can reduce Cr(VI) to Cr (III) through a redox reaction (Park et al. 2005) (Table 15.1).

Algae are also used for bioremediation of heavy metal polluted effluent where living algae are found to be more complex than non-living algae. Living algae absorb heavy metal ions during the growth phase, and it is considered to be an intracellular process; however, the process of sorption illustrates large variations based on their growth phase. Along with this, the growth of algae is also affected by several environmental factors that directly influence biosorption. In contrast, non-living algal cells absorb metal ions on the surface of the cell membrane, and it is considered an extracellular process (Zeraatkar et al. 2016). For example, Tuzen et al. (2009) investigated the potentiality of *Ulothrix cylindricum* in the removal of arsenic ion

Table 15.1 Heavy metal detoxification from metal-contaminated sites by various microorganisms

Microbial species	Microbial class	Bioremediate toxicity of metal	Mechanism used	References
Sargassum fluitans	Phaeophyceae	Au	Biosorption	Niu and Volesky (2000)
Bacillus subtilis	Bacilli			
Penicillium chrysogenum	Eurotiomycetes			
Pilayella littoralis	Phaeophyceae	Al, Cd, Co, Cr, Cu, Fe, Ni, Zn	Biosorption	Carrilho and Gilbert (2000)
Penicillium canescens	Eurotiomycetes	Pb, Cd, Hg, As	Biosorption	Say et al. (2003)
Ecklonia maxima	Phaeophyceae	Cu, Pb, Cd	Biosorption	Feng and Aldrich (2004)
Gigartina salicornia	Florideophyceae	Cd	Biosorption	Hashim and Chu (2004)
Sargassum baccularia	Phaeophyceae			
Oscillatoria angustissima	Cyanophyceae	Cu, Co, Zn	Biosorption	Mohapatra and Gupta (2005)
Ulva reticulata	Ulvophyceae	Cu, Co, Ni	Biosorption	Vijayaraghavan et al. (2005)
Chlorella miniata	Trebouxiophyceae	Cr	Biosorption	Han et al. (2006)
Spirogyra sp.	Zygnematophyceae	Cr	Biosorption	Bishnoi et al. (2007)
Ceramium virgatum	Florideophyceae	Cd	Biosorption	Sari and Tuzen (2008)
Pseudomonas veronii	Pseudomonadaceae	Cd, Zn, Cu	Biosorption	Vullo et al. (2008)
Ulothrix cylindricum	Ulvophyceae	As	Biosorption	Tuzen et al. (2009)
Cladophora hutchinsiae	Cladophoraceae	Se	Biosorption	Tuzen and Sari (2010)
Aspergillus versicolor	Eurotiomycetes	Cr, Ni, Cu	Bioaccumulation	Tastan et al. (2010)
Cystoseira stricta	Phaeophyceae	Pb	Biosorption	Iddou et al. (2011)
Aspergillus fumigatus	Eurotiomycetes	Pb	Biosorption	Ramasamy et al. (2011)
Kocuria flava	Actinomycetia	Cu	Precipitation	Achal et al. (2011)
Burkholderia dabaoshanensis	Betaproteobacteria	Cd	Biosorption	Zhu et al. (2012)
Bacillus cereus	Bacilli	Cr	Enzyme-mediated	Dong et al. (2013)
Acinetobacter sp.	Gammaproteobacteria	Cr	Biosorption	Bhattacharya et al. (2014)
Spirulina platensis	Cyanophyceae	Cu	Biosorption	Anastopoulos and Kyzas (2015)
Spirulina maxima	Cyanophyceae	Cr	Bioaccumulation	Singh et al. (2016a, b)

(continued)

Table 15.1 (continued)

Microbial species	Microbial class	Bioremediate toxicity of metal	Mechanism used	References
Cladophora sp.	Ulvophyceae	Pb, Cu	–	Ojuederie and Babalola (2017)
Spirogyra sp.	Zygnematophyceae	Pb, Cu		
Hydrodictylon sp.	Chlorophyceae	As		
Oedogonium sp.	Chlorophyceae	As		
Rhizoclonium sp.	Ulvophyceae	As		
Aspergillus fumigatus	Eurotiomycetes	Pb		
Rhizopus oryzae MPRO	Mucoromycetes	Cr		
Saccharomyces cerevisiae	Saccharomycetes	Pb, Cd		
Bacillus cereus strain XMCr-6	Bacilli	Cr		
Kocuria flava	Actinomycetia	Cu		
Sporosarcina ginsengisoli	Bacilli	As		
Enterobacter cloacae B2-DHA	Gammaproteobacteria	Cr		
Gemella sp.	Bacilli	Pb, Cr, Cd	Plasmid mediated	Marzan et al. (2017)
Micrococcus sp.	Actinomycetia	Pb, Cr, Cd	Plasmid mediated	
Hafnia sp.	Gammaproteobacteria	Cd	Plasmid mediated	
Bacillus sp.	Bacilli	Cr	Reduction	Ontanon et al. (2018)
Aspergillus niger	Eurotiomycetes	Cr, Hg, Pb, Co	Biosorption	Acosta-Rodriguez et al. (2018)
Pseudomonas fluorescens	Pseudomonadaceae	Cr	Biodegradation	Kalaimurugan et al. (2020)
Bacillus safensis	Bacilli	Cr	Biodegradation	
Lactobacillus plantarum	Bacilli	Ni, Cr	Biosorption	Ameen et al. (2020)
Pseudomonas aeruginosa	Pseudomonadaceae	Cd, Pb	–	Oziegbe et al. (2021)
Klebsiella edwardsii	Gammaproteobacteria	Cd, Pb		

(As III), *Ulva lactuca* in the detoxification of Cd(II) and Pb(II) (Sari and Tuzen 2008) (Table 15.1).

15.4 Role of Plants in Bioremediation

Phytoremediation is another cost-effective and eco-friendly remediation method where plants are used to remove contaminants in the environment. This approach can also minimize the threat of dispersion of contaminant and protects the original ecotype (Awa and Hadibarata 2020). Phytoremediation can convert degraded land to be used for the cultivation of crops; hence, it has economic value also (Awa and Hadibarata 2020). To degrade organic contaminants, plants use mechanisms like phytoextraction, phytostabilization, rhizodegradation, rhizofiltration, phytodegradation, and phytovolatilization while phytostabilization, rhizofiltration, phytoaccumulation, and phytovolatilization are used to degrade inorganic contaminants (Tangahu et al. 2011). Phytoextraction involves the uptake and movement of heavy metals from soil to above-ground parts of the plants via roots. It removes metals like nickel (Ni), zinc (Zn), and copper (Cu) (Ojuederie and Babalola 2017). Like phytoextraction, phytofiltration also involves the accumulation of metal contaminants by the use of roots of plants (rhizofiltration), seedlings (blastofiltration), or excised plant shoots (caulofiltration) from aqueous wastes. Rhizofiltration mainly aims to clean extracted groundwater, surface water, and wastewater with low concentrations of contaminants (Sharma and Pandey 2014). Phytostabilization involves the absorption of heavy metals on plant roots or retention within the rhizosphere that rendering them harmless and prevent these pollutants from spreading in the environment (Ojuederie and Babalola 2017). Phytovolatilization, on the other hand, deals with the conversion of soil contaminants to their volatile form by plants and associated rhizosphere microorganisms and their consequent release into the atmosphere. Degradation of organic contaminants using plant enzymes such as nitroreductases and dehalogenases is called Phytodegradation while phytostimulation deals with the addition of microbial activity to degrade organic contaminants by exudates from plant roots (Ojuederie and Babalola 2017).

15.4.1 Limitations of Phytoremediation

Although phytoremediation is a promising approach to remediate metal-contaminated soil or water, this method suffers from some limitations. The method of phytoremediation applies only to low or moderately contaminated soils where the plant produces a significant amount of biomass. In highly contaminated soil, the toxic effects of contaminates hinder plant metabolism on reducing the biochemical process that is essential for the degradation and/or uptake of the contaminants. Second, the selection of plants for phytoremediation is very important especially

concerning root depth and age (Chirakkara et al. 2016). Generally, the roots of herbaceous species may reach up to 1 m, bushes from 1 to 3 m, and trees up to 10 m. It is reported that phytoremediation is more successful in the top 50 cm^{-1} m layer (Cameselle et al. 2013). The growth of plants is influenced by climatic and hydrologic conditions (Tangahu et al. 2011), and their physiological activities depend on their age. Usually, the roots of a young plant absorb more ions than their older counterparts. The third limitation is related to the uptake and translocation of metals. The metals must be in bioavailable form, and if the metal is tightly bound to the organic portion of the soil, sometimes it may not available to plants. Additionally, the method is slow in comparison to other remediation technologies, and it may take more than 1 year of treatment (Chirakkara et al. 2016).

15.5 Microbe-Assisted Phytoremediation

To overcome the limitations of phytoremediation, recently, microbe-assisted phytoremediation has been used by many researchers (Rathore et al. 2019; Yamaji et al. 2016; Phieler et al. 2015). The metal-tolerant plant growth-promoting microorganisms (MT-PGPMs) have the potential to enhance the biomass production of plants and better tolerance of plants to heavy metals and help in revegetation and restoration of fertility of the metal-contaminated areas (Abou-Shanab et al. 2006). The microbiome can improve the process of phytoremediation through (1) proton (H^+) release that mediated change in soil pH or formation of organo-metal complexes; (2) binding compounds present in the cell (e.g., organic acids, phytochelatins, and amino acids); (3) influencing redox potential through enzyme-mediated transfer; and (4) enhancing microbial activity in the rhizosphere (Sessitsch et al. 2013; Singh et al. 2011). Further, MT-PGPMs induce plant growth directly by secreting enzymes, plant growth-promoting substances, and solubilization of nutrients (Ma et al. 2013). It is reported that by inoculating the effective isolates to the roots of the growing plants, heavy metal accumulation of inoculated plants increased from 66 to 135% in roots and 22 to 64% in the above-ground parts (Anwar et al. 2012).

15.5.1 Mechanisms Behind the Microbe-Assisted Phytoremediation

The plants growing in metal-contaminated areas attract the beneficial metal-tolerant microorganisms to form plant–microbe inter-relationship for better phytoremediation. For that plant releases signals or root exudates (chemotaxis) to their adjoining soil microorganisms (Bulgarelli et al. 2013). As a result, the microbes develop symbiotic/mutualistic associations with plants and live as endophytes or

free-living rhizospheric microbes. Microbes release protons (H^+) and enzymes which help in acidification and electron transfer in the rhizosphere and thus enhance the bioavailability of metal to plants (Ma et al. 2016). MT-PGPMs alter the soil pH by releasing organic acids including gluconic acid, oxalic acid, and malic acid which form complex with insoluble heavy metals and make it soluble and consequently available to plants and microbes (Mishra et al. 2017). In this connection, Kim et al. (2010) have reported that translocation and bioaccumulation of metals are significantly enhanced by citric and oxalic acid, suggesting that these acids could be used as natural chelating agents for better phytoextraction. The release of metal chelators like metallothione, phytochelatin from plant root exudates and MT-PGPMs also contribute to the detoxification of heavy metals. MT-PGPMs release phytohormones such as indoleacetic acid (IAA), cytokinins, gibberellins, abscisic acid that govern the hormonal balance in plants as a response to metal stress (Ma et al. 2016; Ullah et al. 2015). MT-PGPMs produce ACC deaminase enzyme that hydrolyzes ACC which is the immediate precursor of the hormone ethylene in plants to ammonia and α-ketobutyrate and thus reduce the metal stress on lowering the level of ethylene inside the plants (Glick 2014). There is another mechanism adopted by MT-PGPMs under metal stress conditions to enhance plant growth through the production of antimicrobial enzymes (Saima et al. 2013), and polysaccharides (Naseem and Bano 2014) (Table 15.2, Fig. 15.2).

These play a major role to overcome the negative impact of both biotic (fungi or harmful insects) and abiotic stresses (waterlogging, drought, salt stress, and metals toxicity). Hence, MT-PGPMs can speed up phytoremediation and promote plant growth and development by resorting to any one or more of the above mechanisms. For that reason, MT-PGPMs can be effectively utilized in metal-contaminated environments for the phytoremediation. For instance, experiments assessed by Becerra-Castro et al. (2011) have shown that inoculation of Ni-resistant rhizosphere bacteria *Arthrobacter nitroguajacolicus* in Ni hyperaccumulator *Alyssum serpyllifolium* subsp. *lusitanicum* increases the higher translocation and concentration of Ni in the shoot. Similarly, on inoculating *Psychrobacter* sp., SRS8 in *Ricinus communis* and *Helianthus annuus* was found to enhance the phytoextraction and growth of the plants in Ni-contaminated soils (Sessitsch et al. 2013).

Arbuscular mycorrhizal fungal (AMF) colonization in the plant roots also increases heavy metal tolerance capacity of plants in metal-contaminated soils by depositing metals within cortical cells, binding metals to the cell wall or mycelium as well as sequestering them in their vacuole or other organelles, on releasing heat-shock protein and glutathione, precipitating or chelating metals in the soil matrix by producing glycoprotein or making phosphate-metal complexes inside the hyphae, and reducing the strength of metals by heightened root and shoot growth (Emamverdian et al. 2015; Manchanda et al. 2017). In addition to increasing heavy metal tolerance capacity, AMF improves plant growth by different mechanisms through releasing growth-promoting substances, hormones, improving systemic resistance, synergistic interaction with other soil microorganisms, increasing formation and stabilization of soil aggregates (Yao et al. 2005). Interaction of mycorrhizal inoculation (*Glomus mosseae*) with maize growing in HM

Table 15.2 Combination of hyperaccumulator plants and metal-tolerant microbes applied in microbial-aided phytoremediation of metal overburdened soil

Microbial species	Plant species	Bioremediate toxicity of metal	Effect of microbes on plants	References
Variovorax paradoxus	*Brassica juncea*	Cd	Stimulate root elongation	Belimov et al. (2005)
Rhodococcus sp.				
Flavobacterium sp.				
Pseudomonas sp. LK9	*Solanum nigrum*	Cd	Increases uptake of Cd in shoot and root	Sheng et al. (2008)
Enterobacter aerogenes	*Brassica juncea*	Ni, Cr	Strains enhance plant biomass, protein, and chlorophyll content	Kumar et al. (2009)
Rahnella aquatilis				
Microbacterium sp.	*B. napus*	Cu	Increases the root length	He et al. (2010)
Pseudomonas chlororaphis				
Arthrobacter sp.				
Pseudomonas aeruginosa	*Cicer arietinum*	Cr	Enhances dry matter accumulation, symbiotic attributes, grain yield, and protein content of chickpea	Oves et al. (2013)
Enterobacter ludwigii	*Helianthus annuus*	Co, Pb, Zn	Enhances dry matter accumulation	Arunakumara et al. (2014)
Rahnella sp.	*Amaranthus* sp.	Cd	Significant increase in dry weight was observed with various Cd concentrations	Yuan et al. (2014)
Klebsiella oxytoca	*Helianthus annuus*	Co, Pb, Zn	Increases uptake and translocation from root to shoot	Arunakumara et al. (2015)
S. acidiscabies	*Sorghum bicolor*	Cd, Co, Ni, Sr	Increases the phytoextraction	Phieler et al. (2015)
S. tendae				
Rhizophagus irregularis				
Phialocephala fortinii	*Clethra barbinervis*	Cu, Ni, Zn, Cd, Pb	Enhancement, promotion of nutrient uptake	Yamaji et al. (2016)
Rhizodermea veluwensis				
Rhizoscyphus sp.				

(continued)

Table 15.2 (continued)

Microbial species	Plant species	Bioremediate toxicity of metal	Effect of microbes on plants	References
Sphingomonas macrogoltabidus	*Alyssum murale*	Ni	Ni mobilizer, siderophore producer, and phosphate solubilizer; increases Ni uptake in shoots by 17%	Waigi et al. (2017)
Sphingomonas sp.	*Solanum nigrum*	Cd	AA producer, displays ACCD activity; induces heavy metal tolerance to Cd, Zn, Pb, and Cu	
Ensifer adhaerens	*Betula celtiberica*	As	Enhances plant growth and better accumulation of As	Mesa et al. (2017)
Pseudomonas aeruginosa	*Brassica juncea*	Cd	Increases root and shoot biomass	Rathore et al. (2019)
Pseudomonas tolaasii ACC23	*B. napus*	Cd	Increases root and shoot growth and the Cd content in plant	
Achromobacter xylosoxidans	*B. juncea*	Pb, Cu	Increases root and shoot length, fresh and dry weight and improves metal uptake	
Microbacterium sp. G16	*B. napus*	Pb	Increases root elongation of inoculated rape seedlings and total Pb accumulation	
Pseudomonas fluorescens G10	*B. napus*	Pb	Increases root elongation of inoculated rape seedlings and total Pb accumulation	
Pseudomonas sp. RJ10	*B. napus*	Cd	Increases uptake of Cd by plant, enhances shoot and root dry weight	
Bacillus sp. RJ16	*B. napus*	Cd	Increases uptake of Cd by plant, enhances shoot and root dry weight	
Azotobacter chroococcum	*B. juncea*	Pb, Zn, Cu	Increases the removal of Pb, Zn, Cu	
Bacillus subtilis SJ-101	*B. juncea*	Ni	Increases the accumulation of Ni by 1.5-fold and increased plant biomass	
Acaulospora sp.	*Ricinus communis*	Pb	Phytostabilization to ameliorate Pb pollution and decreasing its ecological risk	Gonzalez-Chavez et al. (2019)
Funneliformis mosseae				
Gigaspora gigantea				
Serratia sp.	*Zea mays*	Zn	Zn toxicity reduced and enhanced the plant growth parameters	Jain et al. (2020)

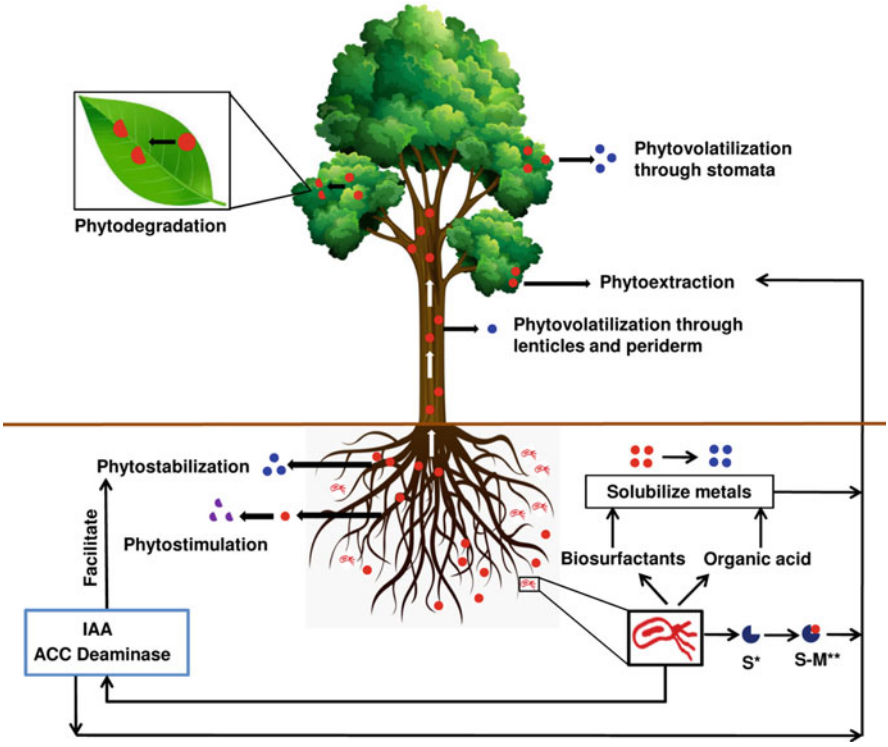

Fig. 15.2 Mechanisms of remediation of heavy metal (HM)-contaminated soil by microbial-aided phytoremediation

contaminated soil showed limiting the metal uptake capacity of the host plant on decreasing the availability of excessive Zn, Cu, and Pb (Huang et al. 2005). AMF colonization influences the production and augmentation of micronutrient uptake capacity of plants grown in heavy metal contaminated soil (Kaewdoung et al. 2016). Oxalate crystals produced by various mycorrhizal fungi (*Fomitopsis* cf. *meliae* and *Ganoderma* aff. *steyaertanum*) are also used to detoxify heavy metals by transforming them to lesser toxic forms such as copper sulfate into copper oxalate hydrate, lead nitrate into lead oxalate, cadmium sulfate into cadmium oxalate trihydrate (Kaewdoung et al. 2016).

15.6 Omics Approaches to Expedite the Remediation Process

Isolation and characterization of the microbial community responsible for bioremediation are imperative; however, with these culture-dependent methods, only 0.1–1% of the soil microbial community can be isolated, leaving more than 99% of microbes either uncultivable or difficult to culture. To overcome these limitations, a range of molecular techniques have been devised to explore the microorganisms responsible for bioremediation (Gupta et al. 2020; Subhashini et al. 2017). It includes fluorescence in situ hybridization technique (FISH), microbial lipid analysis, quantitative PCR, microradiography, stable isotope probing, clone library method, DNA microarray, and different genetic fingerprinting techniques like temperature gradient gel electrophoresis (TGGE), denaturing gradient gel electrophoresis (DGGE), single-stranded conformation polymorphism (SSCP), random amplified polymeric DNA (RAPD), terminal restriction fragment length polymorphism (T-RFLP), ribosomal intergenic spacer analysis (RISA), amplified ribosomal DNA restriction analysis (ARDRA), and length heterogeneity PCR (LH-PCR). All of these methods are based on isolation of lipids, proteins, nucleic acids targeting to amplify genes 16S rRNA, ITS, and 18S rRNA from soil (Gupta et al. 2020). Using these techniques, diversity and variation of the microbial community in contaminated soil in comparison to healthy soil can be analyzed (Panigrahi et al. 2019; Schloter et al. 2018; Malla et al. 2018; Margesin et al. 2011; Yang et al. 2020), but these techniques are unable to provide information about the mechanism involving in the remediation process.

Advanced omics strategies like metagenomics, metaproteomics, metatranscriptomics, and metabolomics provide a comprehensive and profound understanding of the underlying mechanism and adaptation strategy in microbial and plant cells in response to metal stress and thus unlimitedness in their implementation in the remediation of contaminated land (Gupta et al. 2020). Metagenomics provides us to understand not only to explore true diversity of microbes present in diverse environments but also to furnish remarkable information about the genes (*cadB*, *chrA*, *copAB*, *pbrA*, *merA*, *NiCoT*, etc.) responsible to adapt in metal-rich soil on tolerating metal toxicity, so that they can be used for bioremediation (Malla et al. 2018). In that direction, since the last few years, the genome of many metallotolerant bacteria such as *Enterobacter cloacae* B2-DHA isolated from the Hazaribagh tannery areas in Bangladesh, *Geobacillus thermodenitrificans* NG80-2 isolated from a deep oil reservoir in Northern China, *Halomonas zincidurans* strain B6 T isolated from a deep-sea heavy metal-rich sediment from the South Atlantic Mid-Ocean Ridge, *P. putida* ATH-4 isolated from soil sediments at the "Prat" Chilean military base located in Greenwich Island, Antarctica has been sequenced which provides information on the presence of heavy metal resistance genes to survive in the metal-rich environment (Barman et al. 2020). Thus metagenomics-based bioremediation approach is one of the effective tools for the removal of metal toxicity from the environment (Malla et al. 2018).

In response to metal stress, different stress response systems get activated within a given environment, and metatranscriptomics has provided a valuable insight into these gene expressions. Hence, metatranscriptomics is of immense importance for research related to environmental remediation. It was observed that on exposure to high Ni concentration to *Sphingobium*, approximately 118 genes are differentially expressed among which 90 were found to be upregulated (Volpicella et al. 2017). Transcriptome analysis of *E. coli* and *B. subtilis* showed that three membrane stress-related regulons, i.e., *cpxRA*, *rpoE,* and *basRS* get activated in response to metal stress (Hobman et al. 2007). Metaproteomics is suitable to reveal the qualitative and quantitative changes of proteofingerprints in response to metal stress. It reveals the change of physiological profiles in microbes and/or plants that undergo bioremediation. Commonly SDS-PAGE (1D), two-dimensional gel electrophoresis (2-DE), and two-dimensional difference gel electrophoresis (2-D DIGE) isobaric tags for relative and absolute quantitation (iTRAQ) are used by researchers to get information about the change of expression of the protein in response to metal toxicity (Zivkovic et al. 2018; Zhai et al. 2017; Bar et al. 2007). Combining the above-stated tools with mass spectroscopy and de novo sequencing helps to identify the proteins that get expressed on exposure to metals (Lacerda et al. 2007). The changes of proteomics profile in plants on inoculation with plant growth-promoting bacteria (PGPB) for microbe-assisted phytoremediation can also be detected by the metaproteomics approach (Li et al. 2014). However, metaproteomics offers better results in combination with other "omics" approaches. For example, Dore et al. (2015) utilized "omic" approaches with a combination of liquid chromatography and mass spectrometry techniques to identify proteins and extracellular enzymes and analyze fungal responses under various environmental conditions.

Metabolomics is the new entries to the "omics" family that provides information about the cellular metabolic architecture in response to metal stress (Booth et al. 2015). Since microbe and/or plants synthesize several metabolites to adapt metal stress condition, identification and quantification of these metabolites provide a better understanding of the functional role of these metabolites in the microbe and/or plant cells and the underlying mechanism involved in bioremediation (Malla et al. 2018). An example of this is the metabolomics profiling of *P. pseudoalcaligenes* KF707. It was observed that the strain displayed variation in levels of several metabolites with and without tellurite (Tremaroli et al. 2009). Wang et al. (2015) explore the metabolite profiling of radish roots on exposure to lead (Pb) and cadmium (Cd) stress. Results indicate that a large number of metabolites like sugars, amino acids, and organic acids alter in response to metal stress. The metabolite profiling of maize inoculated with PGPB also provide a better understanding of the upregulation of photosynthesis, hormone biosynthesis, and tricarboxylic acid cycle metabolites in maize that help the host plant to remediate metal-contaminated land as well as better growth and development of the plant in metal-contaminated land (Li et al. 2014).

15.7 Use of Genetically Modified Organisms (GMOs) in Bioremediation

GMOs mean "'any living organism that possesses a novel combination of genetic material obtained through the use of modern biotechnology.'" Microbes and/or plants can be genetically modified by recombinant DNA technology to yield a product having a special feature that has received great attention in bioremediation (Gupta and Singh 2017). Despite that, the use of GMOs in field conditions is restricted due to the associated issues of a biological system such as their reach to the contaminants, activity, competition, and most widespread contaminated sites; hence, it is largely limited in the laboratory (Gupta and Singh 2017). The requirement for the development of GMOs for bioremediation of contaminated sites involves four principal approaches. These include modification of enzyme affinity and specificity, construction and regulation of specific pathways, development of bioprocess for remediation and its monitoring and control, use and applications of biosensors for chemical sensing, toxicity reduction, and endpoint analysis (Gupta and Singh 2017). For instance, Dash and Das (2015) constructed a transgenic bacterium *Bacillus cereus* BW-03 (pPW-05) with the introduction of *merA* encoding mercuric reductase from *Bacillus thuringiensis* PW-05 in the other mercury-resistant marine bacterium *B. cereus* BW-03 (pPW-05) for better bioremediation. It was observed that the *Bacillus cereus* BW-03 (pPW-05) improves the mercury removal efficiency in comparison to the parent strains in situ. The strain also found to survive under varied conditions of pH, salinity, and mercury concentration which increase its possibility to use for bioremediation in the mercury-contaminated field. Arsenic is one of the highly toxic metals in oxidized forms, and its bioremediation is mainly associated with volatilization. Though various indigenous microflora have been reported to volatilize arsenic, the efficiency of volatilization was found to be increased by genetically modified microorganisms. Studies have reported that cloning and expression of arsenite S-adenosyl methionine methyltransferase gene (*arsM*) of *Sphingomonas desiccabilis* and *Bacillus idriensis* increase the release of methylated arsenic gas tenfold more than the wild strain (Yang 2010). Further, the introduction of microbial metal resistance genes in hyperaccumulating plants like *Arabidopsis thaliana*, *Brassica juncea*, *Populus angustifolia*, and *Nicotiana tabacum* has been found to enhance metal transformation and accumulation efficiency as compared to wild plant species. For example, the introduction of *merA* and *merB* from bacteria in *Arabidopsis thaliana* leads to an increase in the tolerance capacity of the plant as well as the better conversion of toxic mercury into its less toxic form (Bizily et al. 2000). In another study, it was observed that transformation and overexpression of the phytochelatin synthase (*TaPCS1*) gene in *Nicotiana* resulted in a better tolerance capacity of the plant toward lead (Gisbert et al. 2003).

15.8 Conclusion and Prospects

From the above thorough and critical discussion, it is evident that remediation technologies using microorganisms are more feasible to decontaminate the metal-polluted site with great economical and ecological relevance. Toward a much deeper perceptive and understanding of the microbial and microbe-assisted phytoremediation, it was observed that they employ different mechanisms to survive in the metal-contaminated site and subsequently performing bioremediation. And various omic-approaches provide a significant advantage to understand the mechanisms involved in bioremediation pathways. From the recent research articles, it is evident that MT-PGPR is an effective and sustainable measure for the reclamation of metal-polluted soils. However, in the future, the contribution of genes about Phyto beneficial traits and the occurrence of preferential symbiosis needs to be studied in-depth to harness the benefit of plant–microbe interactions. Additionally, the application of these potential microorganisms as bioinoculants to be explored for better productivity and remediating the metal-contaminated site. Hence, further research is needed to develop novel bioinoculants to tackle the threat of metal-contaminated sites. Additionally, different biotechnological approaches provide an avenue to develop the designed microbes to improve the bioremediation potentiality and better productivity under stress conditions, but in connection with regulatory risk assessment, the field application of GMOs is still restricted. Hence, further improvements in GMOs in terms of their survival, completion with an indigenous population, and chemotaxis toward the pollutants along with structural genes associated with bioremediation of contaminants should also be considered while developing GMOs for environmental cleanup.

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