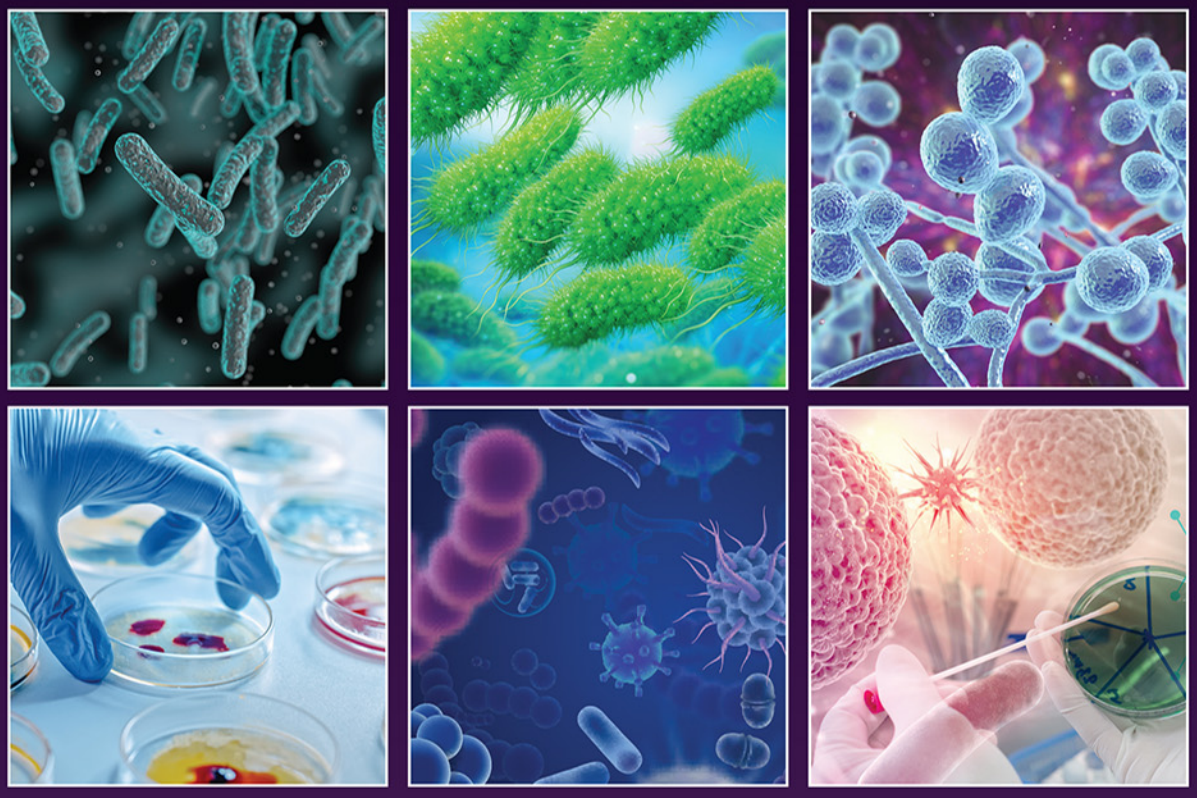
Microbes and Microbial Biotechnology for Green Remediation



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Chapter 34

Microbial remediation of mercury-contaminated soils

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34.1 Introduction

Mercury is a global pollutant that stands among the top ten most toxic chemicals of concern (O'Connor et al., 2019; WHO, 2010). Mercury is released both naturally and anthropogenically. Anthropogenic emissions were estimated to be 2220 tons globally for the year 2015 (O'Connor et al., 2019; UNEP, 2019). Once released, mercury persists in the environment. It may exist in inorganic divalent [Hg(II)] or monovalent [Hg(I)], organic (monomethylated mercury, MeHg, dimethylated mercury, Me_2Hg, and ethylated mercury, EtHg), and elemental [Hg(0)] forms. Elemental mercury is more prevalent in the atmosphere, whereas Hg(II) and MeHg forms are more abundant in the terrestrial and aquatic environments. Elemental mercury may oxidize in the atmosphere and the oxidized, inorganic mercury may be deposited on the terrestrial environment. Inorganic mercury forms salts and other stable complexes with the minerals present in the soil. Organic matter contributes to the conversion of inorganic mercury [Hg(II)] to the toxic organic MeHg form (Teng et al., 2020). In this chapter, elemental mercury will be denoted as Hg(0), divalent mercury and its compounds (unless noted specifically) as Hg(II), monovalent mercury and its compounds (unless noted specifically) as Hg(I), and monomethylated mercury as MeHg.

Plants and aquatic organisms tend to absorb mercury from soil and water, causing it to enter the food chain. Biomagnification of mercury in the food chain may cause serious health effects in top consumers (Wang et al., 2020). Humans are exposed to mercury through inhalation of Hg(0) vapor and ingestion of mercury-contaminated food [Hg(II) or MeHg]. Hg(II) specifically interferes with the enzyme activity, altering the cell metabolism and in turn the body functions (Teng et al., 2020). MeHg is a neurotoxin, immunotoxin, and affects cardiovascular system (Teng et al., 2020). Consumption of mercury-laden food products during pregnancy may adversely affect the neurological development of the child since MeHg can pass through the placenta into the fetus (Teng et al., 2020), and cross the blood−brain barrier (Lohren et al., 2016).

One of the most famous episodes of acute mercury toxicity occurred in the 1950s. Mercury polluted wastewater was released from a chemical industry located in Minamata, Japan, to the Bay. MeHg bioaccumulated in the local aquatic biota which was consumed locally. Several instances of mercury poisoning were then observed in the population. The global nature of mercury pollution and exposure was realized over the following decades, eventually leading to the global Minamata Convention on Mercury. The convention was signed in the year 2017 to reduce global mercury pollution caused by anthropogenic activities (Sakamoto et al., 2020).

Wang, Feng, et al. (2012) have reported that the average background concentration of mercury in soil lies between 0.03 to 0.1 mg $kg^{-1}$with 0.06 mg kg^{-1} as average. In several countries, soil quality guidelines or criteria have been developed that stipulate the usability of soil for a particular purpose. For example, in Australia, a species sensitivity distribution method was applied to the toxicological data collected from different databases and used to determine the ecological investigation level (EIL) as1 mg kg^{-1} (NEPC, 2013). Canada has issued soil quality guidelines (SQG = 2.0−30 mg kg^{-1}) that are derived from the toxicological data for plants, invertebrates, and mammals (Gaudet et al., 1995). Similarly, the US Environmental Protection Agency has recommended inorganic mercury concentration to be 2.3 mg kg^{-1} for residential soils and 350 mg kg^{-1} for industrial soils (USEPA, 2015). The UK Environmental

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Agency has given 170, 80, and 3600 mg kg^{-1} (EA, 2009) as guideline values for residential, allotment, and commercial soils, respectively. The values range from 1 to 3600 mg kg^{-1}, and it has been suggested that soil mercury concentrations that satisfy these guidelines values in some cases may still have deleterious effects on soil health (Mahbub, Bahar, et al., 2017). Availability of mercury in the soil makes it amenable to leaching in water, and reduction to Hg(0) and subsequent evasion to the atmosphere. Both cause transport of mercury to downstream locations. These emissions or releases in one place can lead to pollution and exposure in another place. Thus measures are required for remediation of mercury-contaminated soil at the source location itself.

Remediation technologies for treating soil contaminated with mercury can be categorized as physical, chemical, and biological. Ideally, treatment would mean either "permanent" immobilization of soil mercury (stabilization) or conversion of soil mercury to elemental mercury which is then vaporized and is captured. And, the mercury concentration in soil is brought below a stipulated regulatory value. Physical remediation comprises thermal desorption and electrokinetic removal; chemical remediation comprises chemical stabilization and soil washing; and phyto- and microbial-remediation (bioremediation) comprises methods using plants and microbes for mercury stabilization or removal (Wang et al., 2020).

The present chapter focuses on microbial remediation of mercury-contaminated soil, remediation meaning conversion of Hg(II) to Hg(0), or MeHg to Hg(II) to Hg(0), which should be subsequently captured and sequestered; or accumulated and "stabilized." The basic idea is that the microorganisms in a mercury-contaminated soil are often resistant to mercury toxicity to certain extents and can survive by either utilization or accumulation, of mercury. Thus it may be possible to use these microbial species as biocontrol agents to deal with mercury-contaminated soil. Microbial remediation has been vastly researched and applied for water treatment but microbial remediation of contaminated soil has not been explored to that extent (Mahbub, Bahar, et al., 2017). In this chapter, we review the basic information on microbial transformations of mercury and explore the microbial treatment methods that have been applied for the remediation of mercury-contaminated soil.

34.2 The global mercury cycle

Mercury released from natural and anthropogenic sources cycles reversibly between atmosphere, terrestrial, and aquatic ecosystems. In terrestrial ecosystems it may be sequestered, emitted back to the atmosphere, or run off to aquatic environments. In aquatic environments, it may bioaccumulate, circulate in oceans, or may be reemitted to the atmosphere. Mercury released years ago still persists in the surface reservoirs and will continue to exist for decades even after restricting present-day emissions (Amos et al., 2013). Terrestrial reservoirs of mercury can be divided into three pools (Global Terrestrial Mercury Model; Smith-Downey et al., 2010): fast, slow, and armored. Fast reservoirs included top layer of vegetative soil and soil organic matter. Slow and armored soil pools comprise mineralized carbon deposits at greater soil depths. The top layer of soil is humus-rich that retains mercury from atmospheric deposition and stabilizes it by binding to the organic matter. From there, it can be mobilized as run-off, emitted back to the atmosphere, or buried in deeper mineral reservoirs.

34.2.1 Global use and sources

Mercury is present in the Earth's crust and is periodically released during weathering of rocks (Mahbub, Krishnan, Naidu, Andrews, et al., 2017; Mahbub, Krishnan, Naidu, Megharaj, 2017; Teng et al., 2020). Major anthropogenic activities causing mercury pollution include metal mining, refining and other metallurgical processes (McCarthy et al., 2017; Wang, Feng, et al., 2012), coal combustion (Bourtsalas & Themelis, 2019), gold production (Subhavana et al., 2019), chlor-alkali process (Busto et al., 2011), and the steel, cement, and iron industries (Wang et al., 2016). Zinc sulfide ores are rich in mercury as both metals actively react with sulfide; mercury is thus emitted during zinc smelting. Waste disposal sites also contribute to mercury pollution (Teng et al., 2020).

Mercury is also used in the chemical industry for catalysis and in the medical profession such as dental amalgam (Joy & Qureshi, 2020), topical antiseptics, and in manufacturing of electronic and electrical appliances including switches, fluorescent lamps, short-wave ultraviolet lamps, thermometers, sphygmomanometers, and batteries. Pesticides containing mercury have also historically polluted soil directly (Burger Chakraborty et al., 2013). Further, the intentional use of mercury in artisanal and small-scale gold mining (Ji et al., 2018), recovery of gold from electronic waste and use of coal in paper, cotton, jute, bricks, soft coke, colliery, and fertilizer industries release mercury as a by-product (Burger Chakraborty et al., 2013). Natural and anthropogenic sources contribute 13% and 27%, respectively, to

the present-day mercury deposition, whereas the major portion, 60%, is due to legacy mercury emitted in the past (Amos et al., 2013).

34.2.2 Mercury transformations and transport in the environment

The most abundant and stable form of mercury present in the atmosphere is elemental or metallic mercury [Hg(0)]. The lifetime of Hg(0) in atmosphere is approximately between 6−12 months. This allows Hg(0) to travel long distances before its deposition on soil and water (Xie et al., 2020). 75% of the atmospheric or gaseous mercury is released from fossil combustion that later deposits on soil (Teng et al., 2020).

Oxidation of Hg(0) to Hg(II) in the atmosphere is assisted by bromine, ozone, hypochlorous acid, fluorosulfuric acid, and hydroxyl groups present in fog and moisture in the air (Mahbub, Bahar, et al., 2017). Both dry and wet deposition of mercury occurs from atmosphere to soil and vegetation (Teng et al., 2020; Wang et al., 2020). Atmospheric mercury may be transmitted to the terrestrial ecosystem by stomatal or cuticle uptake by plants or direct deposition to soil. From plants, mercury is transported to soil via litterfall and mercury wash-off from the plant/leaf surface (Blackwell & Driscoll, 2015). Hg(II) on the plant surface may also be photoreduced to Hg(0) that then volatilizes back to the atmosphere (Ariya et al., 2015).

After deposition to soil, Hg(0) and Hg(II) interact with soil mineral and organic matter and undergo transformations. The oxidation of Hg(0) to Hg(I) and Hg(II) occurs at the air−soil interface in the presence of oxygen in the soil (Soares et al., 2015). Hg^{2+} forms thiol complex, HgS, Hg(II)Cl_2, Hg−Cl−OH complex, and HgO. Hg(II) also undergoes methylation (Wang et al., 2020).

The mercury pool in the organic soils has been estimated to be 250−1000 Gg (gigagrams) and is estimated to be increasing due to anthropogenic pollution (Obrist et al., 2018). It has been observed that mercury concentrations are lower in deserts and scrublands due to high reemission to the atmosphere, and higher in vegetation and forestation land due to plant-mediated atmospheric mercury deposition (Obrist et al., 2016, 2018). Higher altitude areas with vegetation and forestation are also reported to have a higher mercury deposition and higher concentration of mercury in soil (Blackwell & Driscoll, 2015).

34.2.3 Speciation and mobility of mercury in soil

Mercury speciation and mobility in soil depends on soil properties. The affinity of mercury toward mineral content and organic matter may lead to its immobilization in soil. Hg(II) forms stable complexes with hydroxyl, chloride, and sulfide functional groups present in soil minerals and organic matter including microbial species (Mahbub, Bahar, et al., 2017). Organic matter containing sulfur forms strong covalent bonds with mercury [Hg(II)] whereas oxygen-containing carboxyl and aromatic groups form weaker bonds (Mahbub, Bahar, et al., 2017). Thereafter, divalent mercury compounds may be methylated. Low soil pH results in the formation of monomethyl mercury and at neutral and alkaline pH conditions, dimethyl mercury is formed. Mercury complexation and methylation in soil reduce the bioavailability for remediation by mercury-resistant bacteria, i.e., reduction of Hg(II) to Hg(0) and the subsequent evasion of Hg(0) (Mahbub, Bahar, et al., 2017; Mahbub, Krishnan, et al., 2016).

Soil properties including pH, cation exchange capacity (CEC), organic matter, minerals, and texture play a major role in mercury sorption (Yin et al., 2018). An increase in soil pH facilitates the sorption of Hg(II). CEC depends on the type and ratio of clay present in the soil. The rice paddy soils are susceptible to high mercury levels because of the high clay content which enhances the cation exchange capacity and thus Hg(II) levels are high. CEC is responsible for nonspecific and reversible binding of divalent mercury resulting in a positive association of CEC with Hg(II) sorption in soil. Mercury binds covalently with oxygen groups present in the clay lattice (O'Connor et al., 2019). Clay with finer particle size has higher mercury levels due to a higher surface area. The presence of other potentially toxic heavy metals do not affect the binding of mercury to the soil (O'Connor et al., 2019). Though organic matter can methylate Hg(II) to MeHg, the introduction of mercury-resistant bacteria and fungi can aid in oxidative and reductive demethylation (Teng et al., 2020).

Water content also plays a major role in the degradation of Hg(II); in saturated soil, anaerobic demethylation of mercury occurs in the presence of mercury resistant bacteria (Zhou et al., 2020). Manganese oxide is a soil nutrient and a source for Mn in plants that regulate redox reactions. MnO oxidizes Hg(0) and affects its mobility. Hg(II)-oxide formed due to oxidation is water soluble and can change speciation based on hydraulic conditions. Microbial methylation is enzymatically driven and can be suppressed by a catalyst, such as manganese oxide, that can reduce the rate of sulfate reduction leading to a decreased methylation (Vlassopoulos et al., 2018).

Mercury polarizability is predominant in the presence of a sulfur group that acts as a soft base. Mercury forms a strong bond with the thiol group and behaves as a polarizable soft acid. HgS is considered the most stable form of mercury and thus sulfur-containing adsorbents are introduced in the soil to immobilize mercury in the soil (O'Connor et al., 2019).

Organic matter comprises the microbial community, the debris of degrading plants and animals, and chemical compounds such as chelators, ligands, and aromatic groups. The organic matter contains chelating agents and ligands that are involved in surface complexation and specific binding with mercury. Mercury binds with organic matter to form complexes with soil minerals, that is, organomineral mercury complex. Organic matter has more affinity toward divalent mercury sorption because of the presence of various functional groups including hydroxyl, carboxyl, aromatic, and thiol groups (O'Connor et al., 2019).

Strong binding of Hg(II) and MeHg with soil can cause less availability of mercury for microbial remediation and the presence of other contaminants may have an impact on microbial metabolism (Antoniadis et al., 2017). Thus abiotic factors play a major role in increasing mercury mobility for bacterial demethylation. Humic acid-rich soils form mercury-humic and mercury-fulvic complexes that are water soluble and thus improve mobility under water-saturated and flooded conditions (Fernández-Martínez & Rucandio, 2014; Liu et al., 2020). Improving mercury mobility makes it bioavailable for demethylation. Mercury bound to high-molecular-weight organic compounds present in the soil are less liable to get methylated due to reduced mercury mobility (Šípková et al., 2016). For soil remediation, acidic pH is maintained to increase the mobility of mercury for sequestration and bioavailability for microbial reduction (Mahbub, Bahar, et al., 2017).

34.3 Microbial-mediated reactions of mercury compounds in soil

Soil has a rich microbial community that comprises bacteria, fungi, archaea, algae, protozoa, and viruses. Cell membranes in both bacterial and fungal cells are made of phospholipids and proteins that regulate material flow in and out of the cell. They also act as defense systems and can adapt their permeability according to their physical and chemical environmental (Hongyan et al., 2003). Figs. 34.1 and 34.2 depict the passive and active transport of mercury in the bacterial and fungal cell, respectively, primarily facilitated by cell membranes. More details are presented in Section 34.3.1 and 34.3.2 below.

The bacterial cell wall comprises peptidoglycan that forms a polysaccharide structure cross-linked with short peptides (Vollmer et al., 2008) and the fungal cell wall comprises chitin, glucan, and glycoproteins (Adams, 2004). These polysaccharides and proteins present on the cell wall comprise carboxyl, hydroxyl, acetate, phosphate, and sulfate ester groups. Extracellular accumulation of mercury is mediated by these functional groups that act as ligands for Hg(II) sequestration. Cell wall components such as chitin and chitosan contain −COOH groups and bind covalently with mercury. Mercury uptake is mediated by several metal transporters and is guided to cytosol (Durand et al., 2020; Falandysz, 2016; Urík et al., 2014). Binding of Hg(II) to these functional groups limits the cellular uptake to reduce cell-associated toxicity. Binding of Hg(II) to the cell surface is a mercury tolerance mechanism exhibited by the microorganisms (Grégoire & Poulain, 2014).

The metabolic activity of a cell is carried out in the cell cytoplasm or cytosol. It comprises nutrients, enzymes, growth factors, and cell organelles. All microbial reactions including mercury methylation, reduction, volatilization, and bioaccumulation occur in the cytosol. Some cell organelles like vacuole may participate in bioaccumulation (Grégoire & Poulain, 2014). Bioaccumulation of Hg(II)S is not reported and is substantially lower than MeHg (Mahbub, Bahar, et al., 2017).

34.3.1 Oxidation

Oxidation of Hg(0) was first reported in chemotrophic bacteria by Holm and Cox (1975). Chemotrophic bacteria were incubated in thre presence of Hg(0), which resulted in growth inhibition. The inhibitory effect was suspected to be associated with formation of Hg(II) (Holm & Cox, 1975). Though the mechanistic details behind the formation of Hg(II) are lacking, Magos et al. have proposed a catalase enzyme-mediated pathway could be responsible for biological oxidation (Magos et al., 1978). Under anaerobic conditions, sulfate- and iron-reducing bacteria were able to oxidize Hg(0) to Hg(II). This oxidation process was found to occur extracellularly in the presence of −SH containing low-molecular-weight compounds located on the cell membrane (Colombo et al., 2013; Hu et al., 2013).

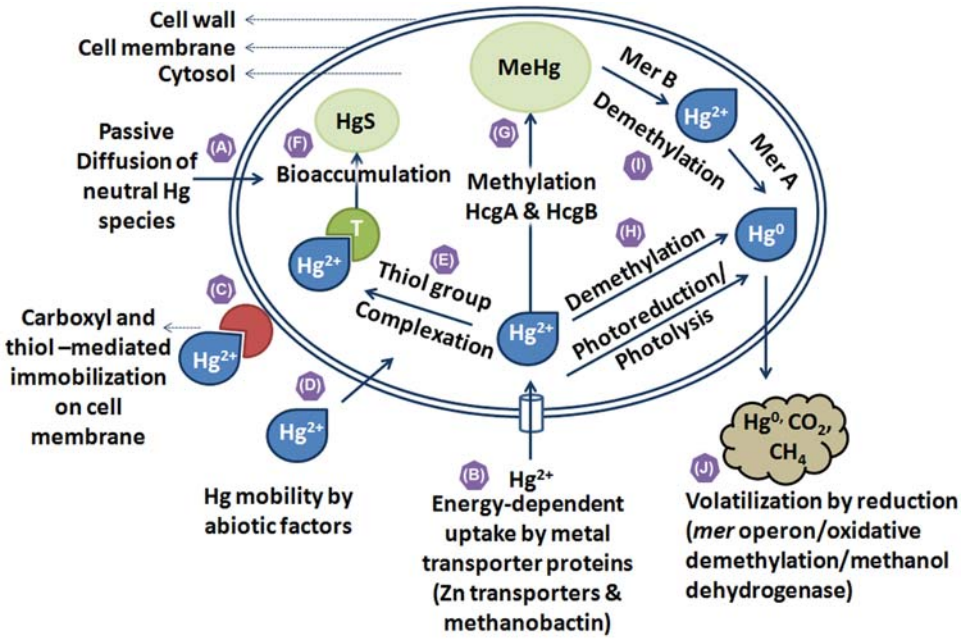

FIGURE 34.1 Bacterial remediation of mercury. Mercury uptake in bacterial cell occurs via two mechanisms, passive diffusion (A) and energy-dependent active transport (B). Mercury ions form complex on the cell membrane by interaction with the thiol, carboxyl and phosphate containing cell membrane constituents (C). Bioavailability of mercury is facilitated in the environment by abiotic factors including, organic content (e.g., humic-acid, low- and high-molecular-weight organic species), mineral content, soil pH, temperature and light/dark conditions (D). In the cytosol, inorganic mercury form thiol complexes (E) and bioaccumulate in the cell (F). In iron- and sulfate-reducing bacteria, inorganic mercury undergoes methylation in the presence of methyl and electron donor proteins, HcgA and HcgB (G). These bacteria carry out oxidative demethylation (H) when the mercury concentration levels are high to reduce cellular toxicity and also possess transporter proteins to export methylated mercury out of the cell. Anaerobic demethylating bacteria, on the other hand, sequester methylated and inorganic mercury species that activates *mer* operon. Organomercurial lyase, MerB, cleaved carbon-mercury bond and mercuric reductase, MerA, catalyzes reduction (I). The Hg(0) escapes the cell (J). Light and dark conditions significantly affect the process by facilitating the electron flow.

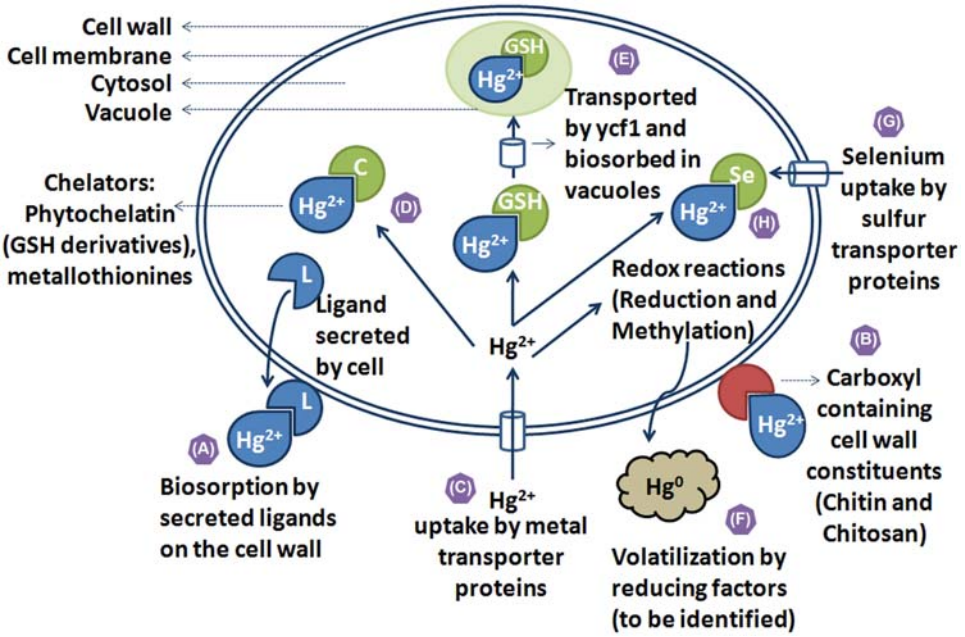

FIGURE 34.2 Mycoremediation of mercury. Extracellular immobilization of mercury is mediated by ligands expressed on the cell wall (A) and the cell wall components containing carboxyl groups (B). Metal transporter proteins assist in mercury uptake (C). Mercury form complexes with thiol compounds (Glutathione (GSH), Phytochelatin, Metallothionines, etc.) and get immobilized intracellularly (D). Mercury-GSH complex is transported to vacuole by ycf1 and is stored there (E). Mercury methylation and reduction mechanisms may be involved that leads to volatilization (F). Selenium uptake is facilitated by sulfur transporters (G). Mercury forms intracellular complexes with selenium and are found to be co-accumulated (H).

34.3.2 Mercury methylation

The organic matter of soil predominantly participates in mercury methylation. Humus-rich soils can act as methyl donors for Hg(II) and cause methylation (Zhu & Zhong, 2015). Under anaerobic conditions, methanogens (Lu et al., 2017), iron- and sulfate-reducing bacteria mediate methylation (Hu et al., 2013), whereas fungal strains (e.g., *Cephalosporium*) methylates mercury in an aerobic environment (Glasauer et al., 2013). A recent in-depth study on mercury methylation has experimentally demonstrated that Hg(II) bound to cell surface does not undergo methylation, whereas mercury transported to periplasmic and cytoplasmic regions gets methylated (Thomas et al., 2020).

An increase in Hg(II) concentration favors methylation and entry into the microbial cell. The two reported mechanisms of Hg(II) uptake include passive diffusion of neutral mercury complexes and the energy-dependent active transport via zinc transporters (Thomas et al., 2018).

34.3.2.1 Passive transport

Passive diffusion can be facilitated by functional groups present in soil mineral. The bacterial cell wall is permeable to Hg(II) neutral species (Fig. 34.1A). Hg(II) interacts with the functional groups, chloride, oxide, and sulfate to form neutral mercury species. Thiol groups (−SH), cysteine (Liu et al., 2016; Schaefer et al., 2014), and glutathione (Liu et al., 2016) have been reported to increase inorganic mercury uptake by the cell for methylation. Inorganic mercury forms sulfide complexes extracellularly where thiol groups act as ligands and also facilitate the transport of methyl mercury out of the cell in anaerobic bacteria such as *Geobacter sulfurreducens* and *Desulfovibrio desulfuricans* (Lin et al., 2015). Similar studies on passive diffusion by fungal cells were not found.

34.3.2.2 Suppression

Lin et al. (2015) studied the impact of thiols on methylating bacteria in the growth medium. The study demonstrated that Hg(II) has a high affinity toward thiols present on cell surface receptors, whereas methyl mercury competes with thiol groups for these receptors. Bacteria (*D. desulfuricans*) self-synthesize thiolate compounds and are efficient methylators and exporters of methyl mercury. Inability to synthesize thiols (*G. sulfurreducens*) can gradually suppress methylation in the cell due to accumulation of methyl mercury in the cell (Lin et al., 2015). Mercury methylation followed by its export from the cell is a survival phenomenon in methylating bacteria. As thiols facilitate methyl mercury transport out of the cell, their absence has a toxic effect on the cells, and mercury methylation is suppressed (Isaure et al., 2020; Lin et al., 2015).

Fungal cells secrete ligands on the cell wall that bind to Hg(II) and immobilize it on the cell surface (Fig. 34.2A). The functional groups present in the fungal cell wall form complexes with Hg(II) (Fig. 34.2B) and cause bioaccumulation (Durand et al., 2020). This suppresses methylation in fungi.

34.3.2.3 Active transport

A study conducted by Schaefer et al. has stated energy-dependent active transport of inorganic mercury by metal transporters (Schaefer et al., 2014), shown in Fig. 34.1B. The presence of high zinc concentration can reduce mercury methylation (Szczuka et al., 2015) but blocking membrane receptor had no effect on methylation rate (Thomas et al., 2020). These transporters facilitate zinc and cadmium uptake. In the presence of mercury, competitive binding occurs that leads to inhibition of zinc and cadmium uptake, increase in mercury uptake subsequently leads to methyl mercury synthesis. The study suggested accidental uptake of mercury by metal transporters (Schaefer et al., 2014). Fungal cells also involve metal transporter proteins for Hg(II) uptake, shown in Fig. 34.2C.

34.3.2.4 Cytosolic process

In iron-reducing bacteria (e.g., *G. sulfurreducens*) and sulfate-reducing bacteria (e.g., *D. desulfuricans*) corrinoid HgcA and ferredoxin HgcB proteins (Schaefer et al., 2014) are involved in the transfer of methyl group and electrons under anaerobic conditions in the cytosol (Pecoraro, 2013), where methylation is carried out (Schaefer et al., 2014), as depicted in Fig. 34.1G. Thiol groups present in cysteine, histidine, and glutathione form complexes with mercury and facilitate accumulation in the cytosol and transport of mercury from the cytosol to cell organelle or to cell wall (Fig. 34.1E) (Schaefer et al., 2014).

Methanogens present in the contaminated soils are also mercury methylators and were found to possess orthologues of HgcA and HgcB proteins (Gilmour et al., 2013; Xu et al., 2019).

Monomethyl mercury has been found to further transform to dimethyl mercury in the presence of dithiol and other sulfur-containing groups. The microbial species responsible for this conversion are to be identified (Jonsson et al., 2016). Baldi et al. (1993) have reported methylation of mercury by *D. desulfuricans* in both monomethyl and dimethyl forms in aqueous conditions and Wallschläger et al. (1995) reported the presence of dimethyl mercury in flooded plain soils.

In fungal cells, intracellular complexation is driven by −SH group-containing chelators present in the cytosol as shown in Fig. 34.2G and H. Selenium enters the cell through membrane transporters and coaccumulates with mercury by forming SeHg complexes (Durand et al., 2020).

34.3.3 Mercury demethylation

Transformation of MeHg to Hg(II) is the demethylation process (Fig. 34.3). Bacterial demethylation can be categorized as *mer*-mediated and *mer*-independent. The following section will explain the *mer* operon and its function.

34.3.3.1 The mer *operon*

Microbial species in mercury-contaminated soils tend to develop resistance against mercury over the years. The genes encoded by the specific genetic regulatory system of bacteria, *mer* operon, was an evolutionary introduction in the bacterial species that functions to provide resistance against mercury (Mahbub, Krishnan, et al., 2016; Mahbub, Krishnan, Naidu, Andrews, et al., 2017; Mahbub, Krishnan, Naidu, Megharaj, 2017; Mathema et al., 2011; McCarthy et al., 2017). The genes are located on chromosomal DNA, transposons, and plasmids that transmit through both horizontal and vertical gene transfer in bacterial species.

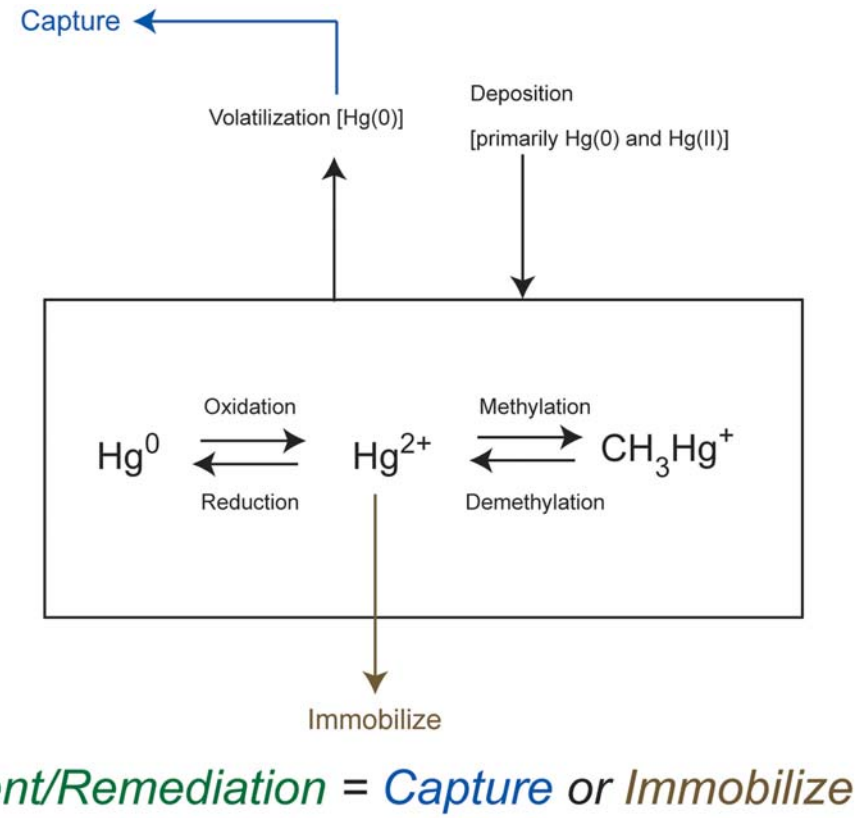

FIGURE 34.3 Redox reactions of mercury species in soil. Atmospheric mercury species (Hg(0) and Hg(II)) on deposition in soil, interact with soil mineral and organic content. Hg(0) oxidizes to Hg(II), which forms neutral species with hydroxyl, chloride, carboxyl and sulfur groups. Hg(II) also gets methylated by methyl donors present in the soil (e.g., humic acid) and by the microbial community. Bacterial demethylation of MeHg to Hg(II) and bacterial reduction of Hg(II) to Hg(0) are the major phenomena involved in remediation. Demethylation is carried out by mercury-resistant bacteria (MRB), fungi, methanogens, iron-reducing bacteria, and sulfate-reducing bacteria. MRB demethylates by implementing reductase and lyase enzymes. Some amount of MeHg and Hg(II) is bioaccumulated in the bacterial, and most gets volatilized.

Fig. 34.1I depicts demethylation via the *mer* operon, harbored by aerobic bacteria for reductive demethylation. Gene products encoded by the *mer* operon have various functions to regulate metabolic activity in bacteria. Some genes have a narrow spectrum to target a specific mercury form, whereas a few can target mercury species broadly. The proteins encoded by these genes function in cytoplasm, periplasm, and inner membrane to facilitate transport, reduction, and lysis of mercury. Genes encoded by the *mer* operon are *merR*, *merT*, *merP, merE*, *merF*, *merC*, *merH*, *merG*, *merA*, *merB*, and *merD* (Mahbub, Bahar, et al., 2017; Naguib et al., 2018).

MerR is a regulatory protein encoded by *merR* gene that binds to the promoter-operator region in the presence and absence of mercury, respectively, to positively and negatively regulate the operon to express other *mer* genes. MerR undergoes conformational changes on interaction with mercury (Mahbub, Bahar, et al., 2017).

The downstream genes present on the operon are expressed when merR binds to mercury. These genes that encode for transporter proteins are *merT*, *merE, merF*, and *merC*. The protein encoded by *merT* transports Hg(II) and organomercury to the inner cell membrane and the uptake of organic and Hg(II) in the cytoplasm is guided by transporter protein of *merE*. Gene product of *merF* is responsible for inner membrane transport. The periplasmic binding of divalent mercury is facilitated by the gene product of *merP*. *merH* also encodes for metallochaperone that distributes mercury to specific cell space, where the metal degrades or accumulates. *merG* located between *merA* and *merB* encodes a protein that provides resistance against phenyl mercury by reducing cellular permeability. The enzymes mercuric reductase and organomercurial lyase are gene products of *merA* and *merB*, respectively. The activation of *mer* operon requires high concentration of bioavailable mercury in soil (Isaure et al., 2020). A few studies suggest oxidative demethylation pathway in absence of organomercurial lyase enzyme (Grégoire & Poulain, 2014).

34.3.3.2 mer-*Mediated demethylation*

Organomercurial lyase translated by *merB* targets broad-spectrum mercury species and cleaves covalent bond between carbon and mercury. For demethylation, merB mediates the protonolytic cleavage of carbon-mercury bond in the cytoplasm and transports Hg(II) to mercuric reductase (Lafrance-Vanasse et al., 2009).

34.3.3.3 mer-*Independent demethylation*

Sulfate-reducing bacteria and methanotrophs could be responsible for oxidative demethylation as CH_4, Hg(II) and carbondioxide were the products identified (Grégoire & Poulain, 2014; Lu et al., 2017; Lu et al., 2016). Methanogens demethylate mercury in absence of *mer* operon by synthesizing methanobactin that binds to methyl mercury and facilitate its transport across the cell as shown in Fig. 34.1B. Methanotrophs utilize one-carbon (C1) as growth substrate by breaking carbon-mercury bond catalyzed by methanol dehydrogenase (Lu et al., 2017) (Fig. 34.1J). Whereas the demethylation mechanism of sulfate-reducing bacteria is currently unclear (Grégoire & Poulain, 2014; Lu et al., 2016).

34.3.4 Reduction

Oxidation of Hg(0) in the aerobic environment occurs via rapid transformation to mercurous (Hg(I)) form which is subsequently oxidized to Hg(II) (O'Connor et al., 2019). Fig. 34.3 shows the oxidation process. The presence of sulfur-containing compounds in the soil mineral and in microbial cells facilitates the synthesis of neutral mercury complexes such as $HgCl_2$ and HgS (sulfur from thiol groups) (Isaure et al., 2020). The uptake mechanism of these complexes and Hg(II) remains obscure (O'Connor et al., 2019; Schaefer et al., 2011).

34.3.4.1 mer-*Mediated reduction*

Aerobic conditions and bioavailability of Hg(II) (in micromolar concentration) activate the *mer* operon. The *mer* operon gene *merA* encodes mercuric reductase enzyme in the cytoplasm that catalyzes Hg(II) to Hg(0) reduction and the volatilized Hg(0) escapes from the cell. Mercuric reductase is a narrow-spectrum enzyme that targets NADPH. At the catalytic site of enzyme, two cysteine residues form complex with Hg^{2+} and NADPH undergoes a hydride (H^+) transfer in the cytoplasm with a simultaneous $FADH^-$ formation. This $FADH^-$ reduces Hg^{2+} and gets oxidized in the process releasing mercury vapor [Hg(0)] (Lian et al., 2014). The volatilized mercury can be captured on porous material (cellulose fibers, activated carbon, etc.) or in a packed-bed bioreactor for immobilization (Wang et al., 2020).

MerR in the presence of mercury ions upregulates the expression of *merA* and is downregulated by MerD on exhaustion of mercury concentration. Oxidase activity of *merA* in the absence of mercury leads to the synthesis of hydrogen peroxide causing cellular toxicity, thus it is important to downregulate the expression of *merA* by MerD (Naguib et al., 2018).

34.3.4.2 mer-Independent reduction

mer-independent reduction of Hg(II) has been observed under anaerobic conditions. Iron-reducing bacteria, *G. sulfurreducens* PCA and *Geobacter metallireducens* GS-15, were found to reduce Hg(II) to Hg(0) in the presence of an electron acceptor, ferric oxyhydroxide. Iron-reducing bacteria have been found to release ferrous ions during growth phase which behave as electron transporter for mercury reduction (Wiatrowski et al., 2006). The fungal strains express homologs of *merA* in response to stress-induced by mercury levels (Chang et al., 2020).

34.4 Microbial treatment of mercury in soil

Transformation of MeHg to Hg(0) by demethylation, followed by reduction and volatilization, and immobilization of Hg(II) compounds such as HgS and other complexes (Fig. 34.2D and E) in the cytosol form the basis of mercury treatment. Commercially used physical and chemical processes for the remediation of mercury-contaminated soil incur high-cost and have a high-energy requirement. These processes also sometimes affect soil fertility (Teng et al., 2020). Microbial-mediated treatment is an eco-friendly process (Kumari et al., 2020) that employs indigenous microbial community to immobilize and demethylate mercury. It is important to understand that during microbial treatment, reductive and oxidative demethylation transforms organomercury to inorganic and elemental mercury, which is more mobile (Zhou et al., 2020). This can increase the bioavailability of mercury in soil that can be biosorbed or adsorbed. Volatilized mercury vapors may escape to the atmosphere and reenter the biogeochemical cycle (Obrist et al., 2018). Thus it is difficult to completely eliminate mercury from the environment. Actinobacteria, Proteobacteria, and Firmicutes are a few mercury-resistant bacterial phyla that possess the *mer* operon (Naguib et al., 2018). Plant growth-promoting bacteria also play a major role in the mobilization of mercury species in soil and contribute toward microbially assisted phytoremediation (Wang et al., 2020). Endophytic fungal species also bioaccumulate mercury and improve resistance to mercury toxicity in plants (Pietro-Souza et al., 2017).

Microbe-assisted reaction mechanisms have been explained in the earlier sections. Treatment by microbial reduction will be referred to as microbial remediation. The following sections will discuss the application of specific microbial species in the remediation of mercury-contaminated soil.

34.4.1 Studies on bacterial remediation

A number of bacterial phyla have been identified for bioremediation, including Firmicutes (genus: *Bacillus*, *Clostridium*, *Enterococcus*), Proteobacteria (genus: *Pseudomonas*, Sphingopyxis, Klebsiella), and Actinobacteria (genus: *Rhodococcus, Mycobacteriaceae*) (Ji et al., 2018; Osborn et al., 1997). Chen et al. (2019) reported 91.6% mercury volatilization by *Bacillus* DC-B2 in Luria broth agar medium with no accumulation (Chen et al., 2019). *Sphingobium* SA2 cultured on phosphate minimal medium showed 80% mercury volatilization in 6 hours (Mahbub, Krishnan, et al., 2016); whereas SE2 was capable of 44% volatilization and 23% accumulation in the cells with no volatilization from dead biomass (Mahbub, Krishnan, Naidu, Andrews, et al., 2017; Mahbub, Krishnan, Naidu, Megharaj, 2017). Bioaccumulation of mercury as HgS is depicted in Fig. 34.1F. In the case of *Sphingopyxis* SE2, 5.97 mg L^{-1} was found to be the mercury concentration that resulted in the death of 50% of the bacterial population in the culture medium (Mahbub, Krishnan, Naidu, Andrews, et al., 2017; Mahbub, Krishnan, Naidu, Megharaj, 2017), which may be due to toxicity caused by MeHg accumulation.

In microcosm under flooded conditions, Hg(II) reduction was observed in natural conditions but the addition of *Bacillus* DC-B2 enhanced mercury volatilization by ~17% in 30 days. The same setup was applied for mercury-contaminated farmland soil and was found to give 82.1% Hg(II) removal in 30 days (Chen et al., 2019).

Another study suggested bioaugmentation of Hg(II) remediation by the introduction of a novel *Pseudomonas* sp. DC-B1 with sawdust biochar. More than 74% mercury volatilization was achieved in the growth medium in 72 hours. In water-saturated microcosm, the addition of biochar enhanced mercury volatilization compared to water-saturated control and addition of *Pseudomonas* sp. DC-B1 further enhanced the remediation by 10% to 23% in Hg(II)-spiked microcosm supplemented with biochar and DC-B1 (Chen, Dong, et al., 2018).

Mercury is strongly bound to clay, minerals and organic matter in the soil, thus is not easily available for remediation. In a study by Chen, Dong, et al. (2018), chemical extraction and bacterial reduction were used to make mercury more bioavailable and amenable for remediation of mercury-contaminated soil. For this two-stage process, ammonium thiosulfate was used to increase the bioavailability of mercury in the soil and *Enterobacter cloacae* B7 was isolated from soil near the pesticide plant in Taiwan. 65% mercury was extracted through the process in 24 hour. *E. cloacae* B7

was able to grow and sustain a high concentration of ammonium thiosulfate (0.5 M) used for extraction. To improve the process, calcium and magnesium ions were added to induce mer operon. Mercury extraction increased to 77% by implication of thiosulfate followed by 81% Hg^{2+} remediation in the presence of calcium and magnesium ions. The two-stage process was checked in the microcosm (Chen, Lin, et al., 2018).

A recent study by Zhou et al. (2020) reported mercury demethylation by the microbial community present in contaminated paddy soils (Zhou et al., 2020). The experiments were conducted in water-saturated microcosm, where molybdate and bromoethane sulfonic acid were used to inhibit the activity of sulfate-reducing bacteria and methanogens, respectively (as these two bacteria are responsible for the conversion of Hg(II) to MeHg). Inhibition of these microorganisms contributed significantly to the inhibition of mercury methylation. Copper was added as a nutrient to enhance the metabolic activity of methanotrophs, naturally present in the contaminated soil, to facilitate demethylation. This study uniquely identified biomarkers involved in demethylation by comparing the microbial taxa present in control and test samples. The mercury transformation-related genes including dsrA, dsrB, mcrA, pmoA, and *hgc*A were amplified using qPCR. The microbial taxa were identified by random forest analysis followed by biomarker identification using linear discriminate effect size analysis. The genes expressed at two time points 0 and 48 hours were compared using DESeq tool. The microbial predictors of mercury demethylation in the contaminated soil were Catenulisporaceae, Frankiaceae, Mycobacteriaceae, and Thermomonosporaceae (Zhou et al., 2020).

Endophytic bacteria or rhizosphere bacteria are responsible for microbially enhancing phytoremediation. These bacterial species change the mobility of mercury species through soil pH modification, redox reactions, and release of chemicals (e.g., chelators). Studies have suggested that microbial consortia can promote plant growth (plant growth-promoting bacteria, PGPB) (Shameer & Prasad, 2018). *Pseudomonas* sp., *P. jessenii*, *Burkholderia* is one such consortium reported to have metal resistance. *Pseudomonas* and *Bacillus* species are among the most abundantly present PGPB and possess biosorption ability. The negatively charged cell membrane of these bacterial species interacts with metal cations and captures them on the cell surface. In a few cases, accumulation has also been observed inside the cells (Shameer & Prasad, 2018). Exopolysaccharides with hexosamines are responsible for mercury removal (Singh & Kumar, 2020).

Metallothioneins present in rhizosphere bacteria have cysteine residues. The thiol group of cysteine binds to metal ions resulting in metal accumulation. Biosorption of metals by PGPB reduces the plant-associated mercury toxicity (Lal et al., 2018). On the other hand, six strains studied by Mello et al. (2020) showed both bioaccumulation and volatilization potential. Serratia and Pseudomonas species increased mercury volatilization by 47.16% and 62.42%, respectively. *Acinetobacter*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, and *Serratia* cause accumulation of mercury in plant tissue and promote the growth of maize (except *Klebsiella*) (Mello et al., 2020).

Methanotrophs are ubiquitously present in the mercury-contaminated soil but have not been studied much for their role in demethylation of mercury. Unlike *mer* operon-mediated demethylation (at mercury micromolar level), methanotrophs can demethylate at even lower mercury concentrations (nanomolar). Lu et al. (2017) reported *Methylosinus trichosporium* OB3b and *Methylococcus capsulatus* Bath as potential methanotrophs. *M. trichosporium* OB3b can biosorb and degrade methyl mercury, whereas *M. capsulatus* Bath can only biosorb and accumulate. Methyl mercury uptake was found to be facilitated by a metal-binding peptide, methanobactin. Methanobactin is originally associated with copper uptake to carry out methanogenesis catalyzed by methane monooxygenase. The presence of copper reduced the uptake of methyl mercury and its demethylation. In absence of methanobactin synthesis, the mutants of *M. trichosporium* OB3b did not demethylate regardless of the same culture conditions (Lu et al., 2017). Interestingly, methanotrophs lacking methanobactin synthesis can ultilize methanobactin produced by other microbes to demethylate mercury and can be a part of syntrophic pathway (Semrau et al., 2019).

Lu et al. (2017) also studied the degradation pathway followed by OB3b by supplementing the nitrate minimal salt culture medium with 5 mM methanol. Methanol is the growth substrate for methanotrophs and in its absence methanotrophs implement alternative pathways. Methanol supplementation reduced demethylation due to competitive substrate inhibition. One-carbon (C1) from methanol was metabolized in substrate supplementation study, whereas in absence of methanol, C1 was supplemented by methyl mercury by cleavage of carbon-mercury bond. This cleavage is catalyzed by methanol dehydrogenase to make C1 available for metabolic pathways. C1 may also be involved in cycling of methyl mercury by other aerobic bacteria (Lu et al., 2017).

Frossard and Hartmann et al. (2017) have studied tolerance in the microbiome of forest soil against high mercury concentration. The study has reported the cooccurrence of bacterial and fungal species. Details on fungal diversity are given in Section 34.4.2. Bacterial α- and β-diversity reduction was observed with a spike in mercury concentration and varied in different soil types. Bacteria with the *mer* operon were more profound in the microcosm showing a shift in bacterial diversity due to mercury-induced stress. Therefore, it is evident that the soil bacterial community adapts according to the environment for survival and growth.

As such, mercury-resistant bacterial species can be added as a biofertilizer to mitigate mercury-toxicity in soils. The overall mechanism of bacterial uptake, methylation, demethylation, bioaccumulation, and volatilization of mercury is shown in Fig. 34.1.

34.4.2 Studies on fungal remediation (mycoremediation)

Remediation of a contaminated zone by fungi is called mycoremediation. The fungal strains are known to adapt to the changed soil environment. Some fungi are hyperaccumulators of mercury that may have a significant impact on mercury cycle (Durand et al., 2020). Bioaccumulation of mercury in fungus is species-dependent and fungal-tissue dependent. More mercury accumulates in the cap than in the stalk. The senescence of the fungus decreases the accumulation efficiency, with the highest potential in the fruiting bodies. Contrary to the bacteria, fungi can bioaccumulate mercury from both soil with high mercury concentrations or low mercury concentrations (Durand et al., 2020). Durand et al. (2020) have also reported possible mechanisms involved in mycoremediation and have been depicted in Fig. 34.2.

Mercury accumulation on the cell wall can be seen in Fig. 34.2A, B, D, and E depict mercury accumulation by complexation in cytosol and transportation of the mercury complex to vacuolar space assisted by ycf1 transporter, respectively (Durand et al., 2020).

Indigenous fungal strains in metal-contaminated soils are metallotolerant and are used for the remediation of soil (Khan et al., 2019). These fungal strains can be cultured, checked for remediation potential in vitro by optimizing the conditions and then applied for ex situ and in situ remediation. *Aspergillus niger* and *Aspergillus terreus* isolated from mercury-contaminated soil of an industrial estate gave >95% mercury removal in the culture medium (Khan et al., 2019).

In general, macrofungi are known for years to biosorb the metal from the contaminated soils (Falandysz, 2017). The fruiting bodies of edible macrofungal strains can accumulate metal and bio-transfer to the food chain. So, macrofungi can be used as bio-indicator and accumulator of metals. *Amanita muscaria* (Falandysz & Treu, 2019), *Boletus edulis* (Kavčič et al., 2019), *Macrolepiota procera* (Falandysz et al., 2017), *Xerocomusbadius* (Kojta & Falandysz, 2016) *and Xerocomus subtomentosus* (Falandysz, 2017), have been reported to efficiently sequester mercury. The mercury uptake can vary from one fungal species to another, based on mercury content and mercury speciation in the soil, and soil physicochemical properties. The presence of chelators such as ethylenediamine tetraacetic acid (EDTA) enhances the efficiency to bioaccumulate metals for *Coprinus comatus* (Cen et al., 2012; Falandysz, 2016) and *Tricholoma lobayense* (Wang, Chen, et al., 2012). Both types of mushrooms, ectomycorrhizal and saprophytic, have the ability to accumulate mercury. Although after reaching the maximum uptake potential, the uptake efficiency may decrease with increasing mercury content in the soil. Mushrooms with deeper mycelia have high mercury concentration in their fruiting bodies. Also, longer mycelia can reach deeper soil layers and reduce mercury concentration in the soil depth by mycelia accumulation (Falandysz & Drewnowska, 2015).

Endophytic fungi associated with various plant species play a role in mercury mobility, uptake, and immobilization (Pietro-Souza et al., 2017). Mercury contamination influences the characteristics and distribution of endophytic fungi in the soil. The endophytic fungi (e.g., *Colletotrichum*) found in the mercury-contaminated zone may or may not have mercury-resistance, based on their lineage. Plants with mercury-resistant fungi have shown better growth in mercury-contaminated soils. So, endophytic fungi can bioaccumulate and can assist the host in phytoremediation (Pietro-Souza et al., 2017).

Another study has reported *mer*-mediated toxicity resistance in *Penicillium* DC-F11, a novel strain isolated from contaminated red soil (Chang et al., 2020). This red soil fungus gave 44% removal by mercury volatilization and 40.62% by biosorption on the cell surface. DC-F11 was found to upregulate *mer*A homolog and *mer*B gene under mercury-stress, similar to bacterial *mer* operon. Also, sulfur metabolism pathway, KEGG was found to be activated as mercury binds to thiol groups. The stress-induced by mercury caused upregulation of genes responsible for the assimilation of sulfate for sulfite production. Sulfite leads to the synthesis of low-molecular-weight thiol compounds such as cysteine, homocysteine and methionine. These thiol compounds bound to cytotoxic substrate activates glutathione synthetase for production of glutathione-S-transferases as detoxification enzymes and five members of this family were also found to be upregulated in the presence of mercury stress. Another pathway activated in response to mercury stress was reactive oxygen species (ROS) induced stress defense and damage repair. The ROS genes were found upregulated on exposure to mercury toxicity (Chang et al., 2020).

Urik et al. isolated *Aspergillus*, *Cladosporium*, *Trichoderma*, and *Alternaria* genera from contaminated and noncontaminated sites to compare the bioaccumulation and biovolatilization efficiency (Urík et al., 2014). Regardless of the site of origin, *Aspergillus* and *Cladosporium* specially were found to volatilize upto 80% of mercury in the culture

medium. Biovolatilization was achieved in 7 days under dark standard laboratory conditions. In the environment, photoprocess is known to assist in mercury remediation but the fungal strains isolated in this study could perform volatilization in dark. Also, the use of dead and live fungal biomass is applicable for metal remediation (Urík et al., 2014).

Fungal species tend to change their genetic structure in response to mercury contamination. The fungal and bacterial community in a specific-soil type is correlated and changes based on environmental stress. Frossard et al. (2017) have experimentally and theoretically analyzed change in genetic biomarkers of soil due to mercury contamination. DNA was isolated from the contaminated soil and was amplified by PCR using bacterial and fungal ribosomal sequences. These amplified strands were sequenced using unidirectional pyrosequencing and the identified genes were quantified by real-time PCR. Target bacterial and fungal sequences were analyzed and the specific regions were selected phylogenetically for comparison of bacterial and fungal segments. These target segments were used for building cooccurrence networks. The nodes of clustered topology given by cooccurrence network define the impact of general traits or soil condition [in this case (Frossard et al., 2017)] with respect to a particular site on the microbial community.

Fungal α- and β-diversity was more resilient in comparison to bacterial diversity against high mercury concentrations and less dependent on soil properties (Frossard et al., 2017). The dominant fungal phyla remained same in contaminated and control soils, whereas the dominant bacterial phyla were different in the test and control. *Basidiomycota* decreased with increase in mercury concentration and was accompanied by an increase in *Zygomycota*. The change in fungal diversity was due to acidic conditions prevalent in soil, thus the shift in fungal diversity was due to soil properties and was not linked to increased mercury concentration. High soil pH or if pH falls out of a certain range feasible for fungal growth may lead to decline of survival and growth of fungus (Durand et al., 2020; Frossard et al., 2017). Several fungal strains, *Agaricus*, *Amanita*, *Boletus*, *Coprinus*, *Lycoperdon*, *Marasmius*, and *Mycena* are considered as bioindicators of mercury pollution (Durand et al., 2020). A little is known about fungal mechanism of volatilization. Sequestered mercury in the fungal cell also gets methylated or reduced to elemental form which may be enzymatically catalyzed or may involve methylating agents (Durand et al., 2020; Falandysz, 2016; Urík et al., 2014).

In summary, both indigenous bacterial and fungal strains have the potential to reduce and volatilize mercury by employing mercury-resistance mechanisms. Mercury remediation is carried out enzymatically in the microbial cells that involve substrate utilization, reduction, biosorption or accumulation in the cells and at the cell surface. The genes and gene products of *mer* operon specifically impart tolerance in the cells. Enzymatic substrate utilization of C1 from methyl mercury further assists in demethylation.

34.5 Impact of mercury toxicity on microorganism

Mercury is found to inhibit soil respiration, enzymatic metabolism, and nitrogen fixation processes. Mercury toxicity may lead to a decline of certain microbial genera and increase in the growth of resistant species. A spectral shift in microbial diversity has been reported (Durand et al., 2020; Mahbub, Krishnan, Naidu, Andrews, et al., 2017; Mahbub, Krishnan, Naidu, Megharaj, 2017).

The presence of mercury in soil not only changes soil morphology but also affects microbial community composition and their cellular functions. Another study on structural shift of agricultural soil microbiome (Salam et al., 2019) has observed a massive decline in genera *Staphylococcus* and *Brachybacterium*. Mercury disrupts the intracellular homeostasis as Hg^{2+} forms covalent bonds with cysteine residues, leading to depletion of antioxidant species. The imbalance in homeostasis of pro- and antioxidant causes oxidative stress. The affinity of mercury toward sulfur and hydroxyl groups also leads to protein denaturation and nucleic acid destruction. Mercury also inhibits cell division, enzyme activities and transcription, and ruptures the cell envelope (comprising cell wall and cell membrane) causing cell death (Khan et al., 2009; Salam et al., 2019).

The presence of mercury hinders the microbial process by inducing stress conditions. The resistance in microorganisms is due to the presence of efflux pumps, enzymatic reactions, cell surface binding, etc., and ability to biomethylate mercury (Boening, 2000; Mahbub, Krishnan, Naidu, Andrews, et al., 2017; Mahbub, Krishnan, Naidu, Megharaj, 2017). The effective concentration of mercury in the microbial cells is quantified by toxicity indicators such as enzyme activities (e.g., dehydrogenase, urease alkaline, and acid phosphatase), nitrification, amount of ATP produced and stored, total microbial population, total nitrogen fixers, microbial biomass carbon, arylsulfatase, methane oxidation, and soil respiration. Mercury concentration gradient, the effect of other pollutants on mercury speciation, control soil, and soil aging are the parameters considered to study the impact of mercury on soil microbial community (Mahbub, Krishnan, Naidu, Andrews, et al., 2017; Mahbub, Krishnan, Naidu, Megharaj, 2017).

High levels of mercury also affect the mycelia growth of mercury-resistant fungal strains. The fungal cells combat cytotoxicity by the expression of specific enzymes and proteins involved in the transport of mercury to cells. These

proteins cause the production of chelators (e.g., metallothionein) to modify the cell permeability (Hindersah et al., 2018). Salam et al. have reported an evident decrease in the archaea domain due to mercury contamination and an increase in the population of certain genera with mercury resistance (Salam et al., 2019). Reduction in mycelia length has also been observed in mycoremediation (Falandysz & Drewnowska, 2015).

Mercury toxicity also activates other cellular pathways for stress defense and damage repair. Upregulation of genes involved in metabolism of thiol compounds and glutathione (Fig. 34.2D) can be observed. Stress-mediated activation of catalase and peroxidase enzymes involved in the production of ROS was found to be upregulated due to mercury toxicity (Chang et al., 2020).

34.6 Benefits and limitations of microbial remediation and future implications

Microbial remediation is stated to be an efficient and less costly method for the treatment of mercury-contaminated soil. It also limits secondary pollution to retain soil properties after treatment (Chen et al., 2019; Yap & Peng, 2019). The microbial species required for treatment are indigenously available and their activity can be enhanced by the addition of basic nutrients including copper, calcium and magnesium (Chen, Lin, et al., 2018; Teng et al., 2020). Bacterial and fungal species with mercury tolerance possess the *mer* operon that translates genes to carry out transport, reduction and lysis of mercury species. The presence of a negatively charged cell surface helps in the biosorption of mercury cations. Especially hexosamine-containing exopolysaccharides (extracellular polysaccharides) are required for mercury binding. Mercury mostly can freely pass through the microbial cell membrane and the transporter proteins can also assist in the process.

Only 1% mercury is freely available for microbial remediation, so, to increase the bioavailability of mercury for remediation, physical or chemical processes can be implemented (Chen, Lin, et al., 2018; O'Connor et al., 2019). Soil washing and chemical extraction are used in various studies to increase its availability in the soil. But the implication of physical and chemical process can increase the cost and release of secondary products. These processes can also interfere with microbial activity and alter soil properties (Chen, Lin, et al., 2018). Thus to increase mercury bioavailability further "green" methods should be investigated. Large-scale implementation of microbial remediation still remains questionable as the soil microflora and soil properties changes with environmental conditions. There are no studies yet on the in situ application of microbial remediation for mercury-contaminated sites. The lab scale and microcosm experiments show promising results, thus in situ application may be explored by taking effective measures to sequester volatilized mercury and to monitor long-term stability.

The role of abiotic factors in biosorption, bioavalibility, and demethylation is to be explored. Humic-acid complexation with mercury has a significant impact on mobility and oxidation of mercury, but more research is required to clearly understand the pathways involved. Oxidative demethylation by mercury methylator bacterial species has been reported but the mechanism of demethylation is unclear.

The introduction of the *mer* operon by genetic modification of microorganisms is an extensive process to achieve successful remediation. It is extremely important to design new methods for the treatment of volatilized mercury post-microbial reduction. Implementation of microbial remediation in association with phytoremediation can provide an alternative for stabilization and immobilization by chemical process. The fate of these phytoextractors and phytoaccumulators needs further investigation as these plants will contain elevated levels of mercury which would again need sequestration.

The challenge of technology transfer from the lab to industrial scale can be solved by a risk assessment of the individual site with its unique soil characteristics and mercury speciation to aid in better implementation of remediation techniques. Biochemical mechanisms involved in microbial remediation are to be further explored to understand the pathways upregulated to sustain in the stress conditions. Microbial remediation can be assisted with emerging nanomaterials (Wang et al., 2020) to improve biosorption potential. The study of microbial and genetic biomarkers for mercury toxicity in soils is limited and thus can be a potential research area.

34.7 Conclusion

This chapter discusses the implementation of microbial remediation to treat mercury-contaminated soils. Dry and wet mercury deposition from atmosphere to soil and leaching from agricultural and industrial activities, disposal of mercury-containing domestic and clinical waste are major sources of mercury pollution. Soil properties including pH, clay, mineral, and organic content play major roles in mercury speciation and mobility. Electrostatic interaction and complexation of mercury with hydroxyl, carboxyl, and thiol groups present in clay, minerals, and organic matter lead to

the immobilization of mercury. Microbial community in the soil has the potential to methylate, demethylate, and reduce mercury. Thiol groups play a major role in deciding the mobility of mercury in the soil and in the microbial cells. The presence of iron-reducing bacteria, sulfate-reducing bacteria, and methanogens methylate divalent mercury, reducing the bioavailability of mercury for remediation (reduction to Hg(0)), whereas many indigenous bacterial and fungal strains demethylate and volatilize mercury in response to the induced-mercury stress. These mercury-resistant microbial communities possess the *mer* operon or its gene homologs to carry out transportation, reduction, and enzymatic lysis of mercury compounds for reducing total mercury concentration and conversion of toxic forms. Methanorophs synthesize methanobactin for the uptake of methyl mercury and degrade it by implication of methanol dehydrogenase.

Bacterial species from Firmicutes, Actinobacteria, and Proteobacteria phyla have been found to be extremely efficient in the volatilization of methyl mercury and divalent ions. PGPB can demethylate and biosorb mercury in intra- and extracellular compartments. On the other hand, mycoremediation was successfully achieved by *Aspergillus*, *Penicillium*, macrofungal, and other endophytic species. The activity of microorganisms can be improved by supplementation of soil with amendments such as biochar, copper, calcium, and magnesium ions. Although, the microbial community can acquire resistance and mercury tolerance, an increase in mercury levels affects the enzymatic functions. Disturbance in microbial metabolism leads to the alteration of soil properties which may affect the soil fertility. Thus further studies are required for increasing bioavailability of mercury by eco-friendly measures and genetic modification may enhance microbial metal tolerance and biosorption ability for effective implementation of microbial remediation. Synthrophic pathways of coexistence and cooccurrence of mercury-methylators and demethylators in tyhe soil microbial community must be studied for in situ application of microbial remediation. Hg(0) released as a by-product of microbial remediation must be sequestered on a substrate to restrict its entry in the mercury global cycle. Plant-mediated mercury adsorption may still release mercury in the long term. Thus mercury accumulation in plants may not be a long-term bioremediation method or would need further development.

References

Adams, D. J. (2004). Fungal cell wall chitinases and glucanases. *Microbiology (Reading, England)*, *150*(7), 2029–2035.

Amos, H. M., Jacob, D. J., Streets, D. G., & Sunderland, E. M. (2013). Legacy impacts of all-time anthropogenic emissions on the global mercury cycle. *Global Biogeochemical Cycles*, *27*(2), 410–421.

Antoniadis, V., Shaheen, S. M., Boersch, J., Frohne, T., Du Laing, G., & Rinklebe, J. (2017). Bioavailability and risk assessment of potentially toxic elements in garden edible vegetables and soils around a highly contaminated former mining area in Germany. *Journal of Environmental Management*, *186*, 192–200.

Ariya, P. A., Amyot, M., Dastoor, A., Deeds, D., Feinberg, A., Kos, G., Poulain, A., Ryjkov, A., Semeniuk, K., & Subir, M. (2015). Mercury physicochemical and biogeochemical transformation in the atmosphere and at atmospheric interfaces: A review and future directions. *Chemical Reviews*, *115*(10), 3760–3802.

Baldi, F., Pepi, M., & Filippelli, M. (1993). Methylmercury resistance in Desulfovibrio desulfuricans strains in relation to methylmercury degradation. *Applied and Environmental Microbiology*, *59*(8), 2479–2485.

Blackwell, B. D., & Driscoll, C. T. (2015). Deposition of mercury in forests along a montane elevation gradient. *Environmental Science & Technology*, *49*(9), 5363–5370.

Boening, D. W. (2000). Ecological effects, transport, and fate of mercury: A general review. *Chemosphere*, *40*(12), 1335–1351.

Bourtsalas, A. T., & Themelis, N. J. (2019). Major sources of mercury emissions to the atmosphere: The US case. *Waste Management*, *85*, 90–94.

Burger Chakraborty, L., Qureshi, A., Vadenbo, C., & Hellweg, S. (2013). Anthropogenic mercury flows in India and impacts of emission controls. *Environmental Science & Technology*, *47*(15), 8105–8113.

Busto, Y., Cabrera, X., Tack, F., & Verloo, M. (2011). Potential of thermal treatment for decontamination of mercury containing wastes from chlor-alkali industry. *Journal of Hazardous Materials*, *186*(1), 114–118.

Cen, F., Chen, L., Hu, Y., & Xu, H. (2012). Chelator-induced bioextraction of heavy metals from artificially contaminated soil by mushroom (Coprinus comatus). *Chemistry and Ecology*, *28*(3), 267–280.

Chang, J., Shi, Y., Si, G., Yang, Q., Dong, J., & Chen, J. (2020). The bioremediation potentials and mercury (II)-resistant mechanisms of a novel fungus Penicillium spp. DC-F11 isolated from contaminated soil. *Journal of Hazardous Materials*, 122638.

Chen, J., Dong, J., Chang, J., Guo, T., Yang, Q., Jia, W., & Shen, S. (2018). Characterization of an Hg (II)-volatilizing Pseudomonas sp. strain, DC-B1, and its potential for soil remediation when combined with biochar amendment. *Ecotoxicology and Environmental Safety*, *163*, 172–179.

Chen, J., Dong, J., Shen, S., Mei, J., & Chang, J. (2019). Isolation of the Hg (II)-volatilizing Bacillus sp. strain DC-B2 and its potential to remediate Hg (II)-contaminated soils. *Journal of Chemical Technology & Biotechnology*, *94*(5), 1433–1440.

Chen, S., Lin, W., Chien, C., Tsang, D. C., & Kao, C. (2018). Development of a two-stage biotransformation system for mercury-contaminated soil remediation. *Chemosphere*, *200*, 266–273.

Colombo, M. J., Ha, J., Reinfelder, J. R., Barkay, T., & Yee, N. (2013). Anaerobic oxidation of Hg (0) and methylmercury formation by Desulfovibrio desulfuricans ND132. *Geochimica et Cosmochimica Acta*, *112*, 166–177.

Durand, A., Maillard, F., Foulon, J., & Chalot, M. (2020). Interactions between Hg and soil microbes: Microbial diversity and mechanisms, with an emphasis on fungal processes. *Applied Microbiology and Biotechnology*, 1–22.

EA. (2009). Soil guideline values for mercury in soil: Science report SCO50021/mercury SGV. Environment Agency. Available from https://www.gov.uk/government/publications/land-contamination-soil-guideline-values-sgvs.

Falandysz, J. (2016). Mercury bio-extraction by fungus Coprinus comatus: A possible bioindicator and mycoremediator of polluted soils? *Environmental Science and Pollution Research*, *23*(8), 7444–7451.

Falandysz, J. (2017). Mercury accumulation of three Lactarius mushroom species. *Food Chemistry*, *214*, 96–101.

Falandysz, J., & Drewnowska, M. (2015). Macro and trace elements in Common Chanterelle (Cantharellus cibarius) mushroom from the European background areas in Poland: Composition, accumulation, dietary exposure and data review for species. *Journal of Environmental Science and Health, Part B*, *50*(5), 374–387.

Falandysz, J., & Treu, R. (2019). Amanita muscaria: Bio-concentration and bio-indicative potential for metallic elements. *Environmental Earth Sciences*, *78*(24), 722.

Falandysz, J., Sapkota, A., Dryżałowska, A., Mędyk, M., & Feng, X. (2017). "Analysis of some metallic elements and metalloids composition and relationships in parasol mushroom Macrolepiota procera. *Environmental Science and Pollution Research*, *24*(18), 15528–15537.

Fernández-Martínez, R., & Rucandio, I. (2014). Total mercury, organic mercury and mercury fractionation in soil profiles from the Almadén mercury mine area. *Environmental Science: Processes & Impacts*, *16*(2), 333–340.

Frossard, A., Hartmann, M., & Frey, B. (2017). Tolerance of the forest soil microbiome to increasing mercury concentrations. *Soil Biology and Biochemistry*, *105*, 162–176.

Gaudet, C., Lingard, S., Cureton, P., Keenleyside, K., Smith, S., & Raju, G. (1995). Canadian environmental quality guidelines for mercury. *Water, Air, and Soil Pollution*, *80*(1–4), 1149–1159.

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., Johs, A., Hurt, R. A., Jr, Bailey, K. L., & Elias, D. A. (2013). Mercury methylation by novel microorganisms from new environments. *Environmental Science & Technology*, *47*(20), 11810–11820.

Glasauer, S. M., Beveridge, T. J., Burford, E. P., Harper, F. A., & Gadd, G. M. (2013). *Metals and metalloids, transformation by microorganisms*. *Reference module in earth systems and environmental sciences*. Elsevier, ISBN 9780124095489.

Grégoire, D. S., & Poulain, A. (2014). A little bit of light goes a long way: The role of phototrophs on mercury cycling. *Metallomics*, *6*(3), 396–407.

Hindersah, R., Asda, K. R., Herdiyantoro, D., & Kamaluddin, N. N. (2018). Isolation of mercury-resistant fungi from mercury-contaminated agricultural soil. *Agriculture*, *8*(3), 33.

Holm, H. W., & Cox, M. F. (1975). Transformation of elemental mercury by bacteria. *Applied Microbiology*, *29*(4), 491–494.

Hongyan, Q., Kai, X., & Hongxun, Z. (2003). Phospholipid fatty acid analysis and its applications in microbial ecology. *Acta Ecologica Sinica*, *23*(8), 1576–1582.

Hu, H., Lin, H., Zheng, W., Tomanicek, S. J., Johs, A., Feng, X., Elias, D. A., Liang, L., & Gu, B. (2013). Oxidation and methylation of dissolved elemental mercury by anaerobic bacteria. *Nature Geoscience*, *6*(9), 751–754.

Isaure, M.-P., Albertelli, M., Kieffer, I., Tucoulou, R., Petrel, M., Gontier, E., Tessier, E., Monperrus, M., & Goñi-Urriza, M. (2020). Relationship between Hg speciation and Hg methylation/demethylation processes in the sulfate-reducing bacterium Pseudodesulfovibrio hydrargyri: Evidences from HERFD-XANES and nano-XRF. *Frontiers in Microbiology*, *11*.

Ji, H., Zhang, Y., Bararunyeretse, P., & Li, H. (2018). Characterization of microbial communities of soils from gold mine tailings and identification of mercury-resistant strain. *Ecotoxicology and Environmental Safety*, *165*, 182–193.

Jonsson, S., Mazrui, N. M., & Mason, R. P. (2016). Dimethylmercury formation mediated by inorganic and organic reduced sulfur surfaces. *Scientific Reports*, *6*, 27958.

Joy, A., & Qureshi, A. (2020). Mercury in dental amalgam, online retail, and the minamata convention on mercury. *Environmental Science & Technology*, *54*(22), 14139–14142.

Kavčič, A., Mikuš, K., Debeljak, M., van Elteren, J. T., Arčon, I., Kodre, A., Kump, P., Karydas, A. G., Migliori, A., & Czyzycki, M. (2019). Localization, ligand environment, bioavailability and toxicity of mercury in Boletus spp. and Scutiger pes-caprae mushrooms. *Ecotoxicology and Environmental Safety*, *184*, 109623.

Khan, I., Ali, M., Aftab, M., Shakir, S., Qayyum, S., Haleem, K. S., & Tauseef, I. (2019). Mycoremediation: A treatment for heavy metal-polluted soil using indigenous metallotolerant fungi. *Environmental Monitoring and Assessment*, *191*(10), 622.

Khan, M. S., Zaidi, A., Wani, P. A., & Oves, M. (2009). Role of plant growth promoting rhizobacteria in the remediation of metal contaminated soils. *Environmental Chemistry Letters*, *7*(1), 1–19.

Kojta, A. K., & Falandysz, J. (2016). Metallic elements (Ca, Hg, Fe, K, Mg, Mn, Na, Zn) in the fruiting bodies of Boletus badius. *Food Chemistry*, *200*, 206–214.

Kumari, S., Jamwal, R., Mishra, N., & Singh, D. K. (2020). Recent developments in environmental mercury bioremediation and its toxicity: A review. *Environmental Nanotechnology, Monitoring & Management*, *13*, 100283.

Lafrance-Vanasse, J., Lefebvre, M., Di Lello, P., Sygusch, J., & Omichinski, J. G. (2009). Crystal structures of the organomercurial lyase merb in its free and mercury-bound forms insights into the mechanism of methylmercury degradation. *Journal of Biological Chemistry*, *284*(2), 938–944.

Lal, S., Ratna, S., Said, O. B., & Kumar, R. (2018). Biosurfactant and exopolysaccharide-assisted rhizobacterial technique for the remediation of heavy metal contaminated soil: An advancement in metal phytoremediation technology. *Environmental Technology & Innovation*, *10*, 243–263.

Lian, P., Guo, H.-B., Riccardi, D., Dong, A., Parks, J. M., Xu, Q., Pai, E. F., Miller, S. M., Wei, D.-Q., & Smith, J. C. (2014). X-ray structure of a Hg^{2+} complex of mercuric reductase (MerA) and quantum mechanical/molecular mechanical study of Hg^{2+} transfer between the C-terminal and buried catalytic site cysteine pairs. *Biochemistry*, *53*(46), 7211−7222.

Lin, H., Lu, X., Liang, L., & Gu, B. (2015). Thiol-facilitated cell export and desorption of methylmercury by anaerobic bacteria. *Environmental Science & Technology Letters*, *2*(10), 292−296.

Liu, Y., Zhi, L., Zhou, S., & Xie, F. (2020). Effects of mercury binding by humic acid and humic acid resistance on mercury stress in rice plants under high Hg/humic acid concentration ratios. *Environmental Science and Pollution Research*, 1−11.

Liu, Y.-R., Lu, X., Zhao, L., An, J., He, J.-Z., Pierce, E. M., Johs, A., & Gu, B. (2016). Effects of cellular sorption on mercury bioavailability and methylmercury production by Desulfovibrio desulfuricans ND132. *Environmental Science & Technology*, *50*(24), 13335−13341.

Lohren, H., Bornhorst, J., Fitkau, R., Pohl, G., Galla, H.-J., & Schwerdtle, T. (2016). Effects on and transfer across the blood-brain barrier in vitro—Comparison of organic and inorganic mercury species. *BMC Pharmacology and Toxicology*, *17*(1), 1−11.

Lu, X., Gu, W., Zhao, L., Haque, M. F. U., DiSpirito, A. A., Semrau, J. D., & Gu, B. (2017). Methylmercury uptake and degradation by methanotrophs. *Science Advances*, *3*(5), e1700041.

Lu, X., Liu, Y., Johs, A., Zhao, L., Wang, T., Yang, Z., Lin, H., Elias, D. A., Pierce, E. M., & Liang, L. (2016). Anaerobic mercury methylation and demethylation by Geobacter bemidjiensis Bem. *Environmental Science & Technology*, *50*(8), 4366−4373.

Magos, L., Halbach, S., & Clarkson, T. (1978). Role of catalase in the oxidation of mercury vapor. *Biochemical Pharmacology*, *27*(9), 1373−1377.

Mahbub, K. R., Krishnan, K., Megharaj, M., & Naidu, R. (2016). Bioremediation potential of a highly mercury resistant bacterial strain Sphingobium SA2 isolated from contaminated soil. *Chemosphere*, *144*, 330−337.

Mahbub, K. R., Krishnan, K., Naidu, R., & Megharaj, M. (2017). Mercury remediation potential of a mercury resistant strain Sphingopyxis sp. SE2 isolated from contaminated soil. *Journal of Environmental Sciences*, *51*, 128−137.

Mahbub, K. R., Krishnan, K., Naidu, R., Andrews, S., & Megharaj, M. (2017). Mercury toxicity to terrestrial biota. *Ecological Indicators*, *74*, 451−462.

Mahbub, K. R., Bahar, M. M., Labbate, M., Krishnan, K., Andrews, S., Naidu, R., & Megharaj, M. (2017). Bioremediation of mercury: Not properly exploited in contaminated soils!. *Applied Microbiology and Biotechnology*, *101*(3), 963−976.

Mathema, V. B., Thakuri, B. C., & Sillanpää, M. (2011). Bacterial mer operon-mediated detoxification of mercurial compounds: A short review. *Archives of Microbiology*, *193*(12), 837−844.

McCarthy, D., Edwards, G. C., Gustin, M. S., Care, A., Miller, M. B., & Sunna, A. (2017). An innovative approach to bioremediation of mercury contaminated soils from industrial mining operations. *Chemosphere*, *184*, 694−699.

Mello, I. S., Targanski, S., Pietro-Souza, W., Stachack, F. F. F., Terezo, A. J., & Soares, M. A. (2020). Endophytic bacteria stimulate mercury phytoremediation by modulating its bioaccumulation and volatilization. *Ecotoxicology and Environmental Safety*, *202*, 110818.

Naguib, M. M., El-Gendy, A. O., & Khairalla, A. S. (2018). Microbial diversity of mer operon genes and their potential rules in mercury bioremediation and resistance. *The Open Biotechnology Journal*, *12*(1).

NEPC, Z. (2013). National environment protection (assessment of site contamination) protection measure 1999 (amended 2013). In: Schedule B1: Guideline on investigation levels for soil and groundwater. Canberra: Office of Parliamentary Counsel. Available from http://www.comlaw.gov.au/Details/F2013C00288/Html/Volume_2.

Obrist, D., Pearson, C., Webster, J., Kane, T., Lin, C.-J., Aiken, G. R., & Alpers, C. N. (2016). A synthesis of terrestrial mercury in the western United States: Spatial distribution defined by land cover and plant productivity. *Science of the Total Environment*, *568*, 522−535.

Obrist, D., Kirk, J. L., Zhang, L., Sunderland, E. M., Jiskra, M., & Selin, N. E. (2018). A review of global environmental mercury processes in response to human and natural perturbations: Changes of emissions, climate, and land use. *Ambio*, *47*(2), 116−140.

O'Connor, D., Hou, D., Ok, Y. S., Mulder, J., Duan, L., Wu, Q., Wang, S., Tack, F. M., & Rinklebe, J. (2019). Mercury speciation, transformation, and transportation in soils, atmospheric flux, and implications for risk management: A critical review. *Environment International*, *126*, 747−761.

Osborn, A. M., Bruce, K. D., Strike, P., & Ritchie, D. A. (1997). Distribution, diversity and evolution of the bacterial mercury resistance (mer) operon. *FEMS Microbiology Reviews*, *19*(4), 239−262.

Pecoraro, V. L. (2013). *Bioinorganic fundamentals and applications: Metals in natural living systems and metals in toxicology and medicine*. Elsevier.

Pietro-Souza, W., Mello, I. S., Vendruscullo, S. J., Silva, G. F. D., Cunha, C. N. D., White, J. F., & Soares, M. A. (2017). Endophytic fungal communities of Polygonum acuminatum and Aeschynomene fluminensis are influenced by soil mercury contamination. *PLoS One*, *12*(7), e0182017.

Sakamoto, M., Itai, T., Marumoto, K., Marumoto, M., Kodamatani, H., Tomiyasu, T., Nagasaka, H., Mori, K., Poulain, A. J., & Domingo, J. L. (2020). Mercury speciation in preserved historical sludge: Potential risk from sludge contained within reclaimed land of Minamata Bay, Japan. *Environmental Research*, *180*, 108668.

Salam, L. B., Shomope, H., Ummi, Z., & Bukar, F. (2019). Mercury contamination imposes structural shift on the microbial community of an agricultural soil. *Bulletin of the National Research Centre*, *43*(1), 163.

Schaefer, J. K., Szczuka, A., & Morel, F. O. M. (2014). Effect of divalent metals on Hg (II) uptake and methylation by bacteria. *Environmental Science & Technology*, *48*(5), 3007−3013.

Schaefer, J. K., Rocks, S. S., Zheng, W., Liang, L., Gu, B., & Morel, F. M. (2011). Active transport, substrate specificity, and methylation of Hg (II) in anaerobic bacteria. *Proceedings of the National Academy of Sciences*, *108*(21), 8714−8719.

Semrau, J. D., Kang-Yun, C., Liang, X., Lu, X., Dershwitz, P., Scheper, A., Flatley, A., Lichtmanneger, J., Zischka, H., & Karthikeyan, O. (2019). Methylmercury degradation by methanotrophs and its environmental implications. *AGUFM2019: B42C-06*.

Shameer, S., & Prasad, T. (2018). Plant growth promoting rhizobacteria for sustainable agricultural practices with special reference to biotic and abiotic stresses. *Plant Growth Regulation*, *84*(3), 603–615.

Singh, S., & Kumar, V. (2020). Mercury detoxification by absorption, mercuric ion reductase, and exopolysaccharides: A comprehensive study. *Environmental Science and Pollution Research*, *27*(22), 27181–27201.

Šípková, A., Száková, J., Hanč, A., & Tlustoš, P. (2016). Mobility of mercury in soil as affected by soil physicochemical properties. *Journal of Soils and Sediments*, *16*(9), 2234–2241.

Smith-Downey, N. V., Sunderland, E. M., & Jacob, D. J. (2010). Anthropogenic impacts on global storage and emissions of mercury from terrestrial soils: Insights from a new global model. *Journal of Geophysical Research: Biogeosciences*, *115*(G3).

Soares, L. C., Egreja Filho, F. B., Linhares, L. A., Windmoller, C. C., & Yoshida, M. I. (2015). Accumulation and oxidation of elemental mercury in tropical soils. *Chemosphere*, *134*, 181–191.

Subhavana, K., Qureshi, A., & Roy, A. (2019). Mercury levels in human hair in South India: Baseline, artisanal goldsmiths and coal-fired power plants. *Journal of Exposure Science & Environmental Epidemiology*, *29*(5), 697–705.

Szczuka, A., Morel, F. M., & Schaefer, J. K. (2015). Effect of thiols, zinc, and redox conditions on Hg uptake in Shewanella oneidensis. *Environmental Science & Technology*, *49*(12), 7432–7438.

Teng, D., Mao, K., Ali, W., Xu, G., Huang, G., Niazi, N. K., Feng, X., & Zhang, H. (2020). Describing the toxicity and sources and the remediation technologies for mercury-contaminated soil. *RSC Advances*, *10*(39), 23221–23232.

Thomas, S. A., Mishra, B., & Myneni, S. C. (2020). Cellular mercury coordination environment, and not cell surface ligands, influence bacterial methylmercury production. *Environmental Science & Technology*, *54*(7), 3960–3968.

Thomas, S. A., Rodby, K. E., Roth, E. W., Wu, J., & Gaillard, J.-F. O. (2018). Spectroscopic and microscopic evidence of biomediated Hgs species formation from Hg (II)–cysteine complexes: Implications for Hg (II) bioavailability. *Environmental Science & Technology*, *52*(17), 10030–10039.

UNEP, U.N.E.P. (2019). Global mercury assessment 2018, United Nations Environment Programme, Chemicals and Health Branch Geneva.

Urík, M., Hlodák, M., Mikušová, P., & Matúš, P. (2014). Potential of microscopic fungi isolated from mercury contaminated soils to accumulate and volatilize mercury (II). *Water, Air, & Soil Pollution*, *225*(12), 2219.

USEPA. (2015). Regional screening level (RSL) summary table (TR = 1E-6, HQ = 1) June 2015 (Revised), USA.

Vlassopoulos, D., Kanematsu, M., Henry, E. A., Goin, J., Leven, A., Glaser, D., Brown, S. S., & O'Day, P. A. (2018). Manganese (iv) oxide amendments reduce methylmercury concentrations in sediment porewater. *Environmental Science: Processes & Impacts*, *20*(12), 1746–1760.

Vollmer, W., Joris, B., Charlier, P., & Foster, S. (2008). Bacterial peptidoglycan (murein) hydrolases. *FEMS Microbiology Reviews*, *32*(2), 259–286.

Wallschläger, D., Hintelmann, H., Evans, R., & Wilken, R.-D. (1995). *Volatilization of dimethylmercury and elemental mercury from River Elbe floodplain soils*. *Mercury as a global pollutant* (pp. 1325–1329). Springer.

Wang, D., Chen, L., Jing, X., & Xu, H. (2012). Chelator-and surfactant-assisted remediation of heavy metal contaminated soil by Tricholoma lobayense Heim. *Chinese Journal of Applied & Environmental Biology*, *18*(1), 100–107.

Wang, F., Wang, S., Zhang, L., Yang, H., Gao, W., Wu, Q., & Hao, J. (2016). Mercury mass flow in iron and steel production process and its implications for mercury emission control. *Journal of Environmental Sciences*, *43*, 293–301.

Wang, J., Feng, X., Anderson, C. W., Xing, Y., & Shang, L. (2012). Remediation of mercury contaminated sites—A review. *Journal of Hazardous Materials*, *221*, 1–18.

Wang, L., Hou, D., Cao, Y., Ok, Y. S., Tack, F. M., Rinklebe, J., & O'Connor, D. (2020). Remediation of mercury contaminated soil, water, and air: A review of emerging materials and innovative technologies. *Environment International*, *134*, 105281.

WHO. (2010). *Ten chemicals of major public health concern* (pp. 1–4). World Health Organization.

Wiatrowski, H. A., Ward, P. M., & Barkay, T. (2006). Novel reduction of mercury (II) by mercury-sensitive dissimilatory metal reducing bacteria. *Environmental Science & Technology*, *40*(21), 6690–6696.

Xie, F., Dong, K., Wang, W., & Asselin, E. (2020). Leaching of mercury from contaminated solid waste: A mini-review. *Mineral Processing and Extractive Metallurgy Review*, *41*(3), 187–197.

Xu, J., Buck, M., Eklöf, K., Ahmed, O. O., Schaefer, J. K., Bishop, K., Skyllberg, U., Björn, E., Bertilsson, S., & Bravo, A. G. (2019). Mercury methylating microbial communities of boreal forest soils. *Scientific Reports*, *9*(1), 1–13.

Yap, C. K., & Peng, S. H. T. (2019). Cleaning up of contaminated soils by using microbial remediation: A review and challenges to the weaknesses. *American Journal of Biomedical Science & Research*, *2*(3), 126–128.

Yin, D., He, T., Yin, R., & Zeng, L. (2018). Effects of soil properties on production and bioaccumulation of methylmercury in rice paddies at a mercury mining area, China. *Journal of Environmental Sciences*, *68*, 194–205.

Zhou, X.-Q., Hao, Y.-Y., Gu, B., Feng, J., Liu, Y.-R., & Huang, Q. (2020). Microbial communities associated with methylmercury degradation in paddy soils. *Environmental Science & Technology*, *54*(13), 7952–7960.

Zhu, D., & Zhong, H. (2015). Potential bioavailability of mercury in humus-coated clay minerals. *Journal of Environmental Sciences*, *36*, 48–55.