

Microbial Assisted Phytoremediation
for Heavy Metal Contaminated Soils **16**

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Abstract

Increasing water crisis across the globe, farmers are forced to use marginal quality water for agricultural activities mainly for crop production. Marginal quality water contains lots of contamination load, i.e. microbial population, heavy metals; and caused a range of diseases through food chain contamination. The long- term application of contaminated water accumulate significant amount of heavy metals mostly in industrial regions as well as peri-urban area in developing countries. Use of various phytoremediation technologies for the removal of organic and inorganic pollutant from soil and water are used across the earth boundaries. Among all, bioremediation is a cheaper and more viable technology for the removal of contaminants from contaminated sites. Phytoremediation is a viable, low cost and green technology having a slow process of metal remediation and affecting by the climatic conditions of a particular region. In this regards, use of soil microbial biomass for the decontamination of heavy metals and other contaminated load from soils. The plant-microbe- modulated phytoremediation enhancing the heavy metal remediation, detoxification and mediated the plant nutrient dynamics in a sustainable manner. The soil organic matter decomposition and biogeochemical cycles of plant nutrients are mainly governed by the rhizospheric biomass of the soil. Microbial assisted phytoremediation is a holistic novel approach for the remediation of contaminants. It can use for the location specific contaminant, easy to operate, eco-friendly in nature. In this chapter,

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described the role and interaction effect of plant assisted microbes in heavy metal removal from contaminated soils.

16.1 Introduction

Increasing the production from limited land is forced the farmers to over exploitation of natural resources. These resources, natural resources, i.e. land, water are having its optimum productivity levels and also observed decline rate in agricultural productivity. It is a clear indication of over exploitation of natural resources (Budnuka et al. [2015;](#page-15-0) Singh et al. [2017](#page-21-0)). Most of the developing countries are using poor quality of resources with quantum growth in population (Dotaniya et al. [2016a](#page-16-0), [2017c](#page-16-1)). This situation will be worst in the coming year. A huge volume of industrial and household effluent is generating and contaminating water bodies and land (Saha et al. [2017a\)](#page-20-0). Poor quality water is used for the cultivation of crops; accumulated significant amount of metals in soil (Bingham et al. [1986](#page-15-1); Dotaniya et al. [2014g;](#page-16-2) Singh et al. [2016b](#page-21-1)) and reached to human and animal body via food chain contamination (Hapke [1996](#page-17-0); Fischer [2005](#page-17-1); Rajendiran et al. [2015](#page-20-1); Coumar et al. [2016a,](#page-15-2) [b\)](#page-15-3). Heavy metals are toxic in nature and having an atomic density greater than 5 g cc^{-1} or atomic number more than calcium (Singh [2002](#page-21-2); Emamverdian et al. [2015;](#page-17-2) Rajendiran et al. [2018\)](#page-20-2). In most of the cases trace metals (chromium-Cr, cadmium-Cd, arsenic-As, lead-Pb, mercury-Hg, selenium-Se, aluminum-Al; and essential plant nutrients zinc-Zn, copper-Cu, manganese-Mn) are causing various types of malfunctions in biological system and extreme side caused death (Hapke [1991;](#page-17-3) Dotaniya et al. [2014d;](#page-16-3) Lenka et al. [2016;](#page-18-0) Saha et al. [2017b\)](#page-20-3). The toxicity depends on the type and concentration present in ecosystems (Tchounwou et al. [2012;](#page-21-3) Saha et al. [2017c](#page-20-4)). As per the guidelines issued by the Commission of the European Community regarding the heavy metal permissible limit in dry agricultural soils, i.e. Hg (1–1.5 mg kg⁻¹), Pb (50–300 mg kg⁻¹) and Zn(150–300 mg kg⁻¹) (CEC [1986\)](#page-15-4). In plants, poor growth with toxicity symptoms and in soil reduced the soil biological diversity by the heavy metals (Singh et al. [2011\)](#page-21-4). Application of Cr more than 20 mg kg⁻¹ reduced the germination, root and shoot growth in wheat (Dotaniya et al. [2014a](#page-16-4)) and pigeonpea (Dotaniya et al. [2014c\)](#page-16-5). Increasing concentration of Cr reduced the C mineralization rate and enzymatic activities in Vertisol of central India (Dotaniya et al. [2017d\)](#page-17-4). The enzymatic activities are showing the good bioindicators against reflecting the human disturbance in soil ecology (Hinojosa et al. [2004\)](#page-18-1). It is easy to measure soil quality via soil enzymatic activities in cheaper cost (Khan et al. [2007\)](#page-18-2). These toxicity symptoms are well acknowledged by various researchers in different ecosystems (Malley et al. [2006](#page-19-0); Oliviera and Pampulha [2006](#page-20-5); Wang et al. [2008;](#page-21-5) Saha et al. [2017c](#page-20-4)). Many researchers were described the heavy metal toxicity in term of ED_{50} value, means the metal concentration that inhibited 50% reaction rate of enzymes (Huang and Shindo [2000](#page-18-3)).

Remediation of heavy metals from the soil for the sustainable crop production is a demand of the present situation to combat food shortage for a burgeoning population (Dotaniya et al. [2018c](#page-17-5)). Most of the countries across the world are more focusing on safe utilization of poor resources for mitigating the food, fodder and related demand (Emamverdian et al. [2015;](#page-17-2) Saha et al. [2017a\)](#page-20-0). Use of different remediation methods, i.e. physical, chemical and biological; among all, biological method is cheaper and eco-friendly (Dotaniya et al. [2014d](#page-16-3)). Phytoremedition process using green plants for removing heavy metals from soil water bodies. In which, plant performed various process metabolic and physiological process to decontaminate or removal of process (Singh and Fulekar [2012](#page-21-6)). Plant secreted a range of low molecular organic acids, which degraded the toxic compounds, immobilized, convert toxic to non- toxic, enhanced uptake are pattern of metals (Dotaniya et al. [2013a](#page-16-6), [b](#page-16-7), [d](#page-16-8); Dotaniya and Meena [2013\)](#page-15-5). The plant also converted few metals into volatile compounds and release into the environment (Razzaq [2017](#page-20-6)). In this line, plant associated microorganisms are also performing a valuable place in remediation of metals (Mandal et al. [2017\)](#page-19-1). Plant secreted organic compounds are the source of food for the microbial population of soil. It enhances the microbial count and diversity in soil and accelerates the remediation process (Dotaniya and Meena [2017\)](#page-15-6). It also secreted various types of plant growth promoting substances, and enhanced the growth of the plant in adverse conditions. Plant secreted phytosiderophores are also enhancing the Fe and Zn concentration in soil under deficiency conditions (Dotaniya et al. [2013a\)](#page-16-6). These situations are more favorable for the biological remediation of metals from soil and water bodies. Microbes reduce the toxicity of metal by decomposition or immobilizing the metals from the soil (Abou-Shanab et al. [2003](#page-14-0); Seshadri et al. [2015\)](#page-20-7). In this chapter, most of the microbial assisted phytoremedition mechanisms are described for remediation of metal to enhance the sustainable crop production.

16.2 Heavy Metals Toxicity and the Environment

Heavy metals are metal and metalloid having the high atomic density and trace concentration caused adverse effects on plant, animal and human system (Table [16.1](#page-3-0)). In recent years, metal toxicity pays more attention towards public health and its remediation from ecosystems. The metal toxicity rose due to geogenic and anthropogenic origin and the second one playing more drastic effect on soil and water contamination (SrinivasaGowd et al. [2010](#page-21-7)). The point source of metal toxicity is from mining and extracting of metals, smelting, industrial use and foundries (Fergusson [1990;](#page-17-6) Bradl [2002](#page-15-7); He et al. [2005\)](#page-18-4). Several ways contaminants affected the soil-plant-human continuum on earth and caused toxicity symptoms. Environmental contaminations deteriorate the quality of the environment and affect the ecological process and services (Wenzel et al. [1999](#page-22-0)). Most of the contamination due to atmospheric dry and wet depositions, soil erosion and leaching of heavy metal ions, evaporation of metals via volatilization compounds, trace metal corrosion, automobile exhausts, sewage sludge application and direct contribution from the geogenic origin of metals (Nriagu [1989](#page-19-2); Khan et al. [2007](#page-18-2); Yang et al. [2002,](#page-22-1) [2006](#page-22-2); Kamal et al.

Metals	Major source	Effect on human health
As	Geogenic process, smelting operations, thermal power plants, agricultural inputs (pesticides, fungicides)	It is having chemical structure similar to phosphorus and affect the cell activities, mediated ATP process, bronchitis, skin allergy, poisoning
C _d	Zn smelting, paint industries, e-waste, welding, electroplating, pesticides, fertilizers, batteries industries etc	Carcinogenic, renal dysfunction, mutagenic, Ca imbalance, long-term anemia, lung cancer, kidney damage, gastrointestinal disorder, enzymatic disorder
Ph	Lead acid batteries, paints industrial effluent, coal based thermal power plants, automobile industries ceramics, bangle industries, agricultural chemicals	More toxic to infants, poor development of mental in children, damage nerve system, long exposure caused liver, kidney, gastrointestinal cancer, cardio- vascular disease
Hg	Chlor-alkali industries effluent, pesticides, fluorescent lamps, batteries, medical waste, paper industry, electrical appliances.	Fatigue, hair fall, tremors, memory loss, damage kidney and lungs, damage to nervous system, protoplasm poisoning
Cr	Leather industries, industrial coolants, mining, wooden industries	Hair fall, vomiting, fatigue, skin irritation, damage to the nervous system, eye irritation, long exposure caused cancer
Zn	Agriculture fertilizers, sewage sludge, smelting, electroplating, brass manufacture, plumbing	Vomiting, damage to nerve system, skin irritation, weakness
Cu	Cu mining, pesticide formulations, sulphuric acid plant, chemical industry, metal piping, smelting operations	Brain, liver and kidney damage, chronic anemia, stomach irritation, fatigue

Table 16.1 Source and effect of heavy metals on human health

[2010](#page-18-5)). Increasing the application of metal contaminants in soil or water bodies enhanced the metal concentration in a system. Chromium is the twenty-first most abundant element in the earth's crust, and one of the toxic metals in the environment (Eliopoulos et al. [2013\)](#page-17-7). Land and water pollution by Cr is a worldwide issue. In Western Europe, 1,400,000 sites were affected by heavy metals, of which, over 300,000 were contaminated, and the estimated total number in Europe could be much larger, as pollution problems increasingly occurred in Central and Eastern European countries specially Cr pollution. In the USA, there are 600,000 brown fields which are contaminated with heavy metals and need reclamation (Bahafid et al. [2013\)](#page-14-1). In India, Cr pollution emerged as a challenge to remediate it. It mainly occurs in tannery and paint industries locations. It occurs in nature in bound forms that constitute 0.1–0.3 mg kg^{-1} of the earth's crust (Dotaniya et al. [2014d](#page-16-3)). It has several oxidation states ranging from Cr (-II) to Cr (+VI). It exists predominantly in the Cr^{+3} and Cr^{+6} oxidation states (Dotaniya et al. $2017c$). The most stable oxidation state of Cr is +III, and under most prevailing environmental conditions Cr (VI) is rapidly reduced to Cr (III) (Dotaniya et al. [2014d](#page-16-3)). The intermediate states of +IV and +V are metastable and rarely encountered (Lokhande et al. [2011](#page-18-6)). Application of tannery industrial effluent for crop production accumulated Cr concentration 25–30 times more compared to tube well irrigated fields (Dotaniya et al. [2014g\)](#page-16-2). Such types of studies showing the metal toxicity due to anthropogenic activities and its toxicity effect on soil and plant system. Immobilization of Cr in the plant vacuole of plant roots are the main reasons of Cr concentration higher in plant root than shoot (Oliveira [2012](#page-20-8); Nematshahi et al. [2012\)](#page-19-3). Similar type higher concentration of Ni in soil reduced the uptake mechanism of Fe and Zn; and showed chlorosis symptoms on leaves (Khan and Khan [2010\)](#page-18-7). In plant, metal toxicity affects the plant nutrient mineralization rate and release kinetics in soil and ultimately reduced the plant growth (Singh et al. [2016a](#page-21-8)). Crop plant looks like brushes and crop yield decline drastically. Heavy metal contamination reduced the soil enzymatic activities and carbon mineralization rate (Dotaniya et al. [2017d\)](#page-17-4). Increasing concentration of Cd (2 mg kg^{-1}), in more than 100 mg kg^{-1} Cr contaminated soil; reduce the Cr uptake in spinach crop (Dotaniya et al. [2017a\)](#page-16-9). Zinc toxicity caused induces chlorosis due to deficiency of Fe and Mn in plant (Sivasankar et al. [2012\)](#page-21-9). The deficiency and toxicity of a metal also affected by soil texture, organic matter, soil pH, and concentration of other metals in soil (Bucher and Schenk [2000](#page-15-8); Broadley et al. [2007](#page-15-9); Aref [2011;](#page-14-2) Dotaniya et al. [2014d,](#page-16-3) [2017a](#page-16-9)). Most of cationic heavy metals are more available in lower pH conditions (Dotaniya and Meena [2013](#page-15-5)). Higher concentration (150–300 mg kg^{-1}) of plant essential Zn behaves like toxic metal and reduced the plant growth (Yadav [2010](#page-22-3)).

16.3 Mechanism of Metal Tolerance in Plants

Application of heavy metals produced stress on plant and plant react adversely to counter the negative effect of heavy metal. In this condition secreted various types of secondary metabolites and avoid the harmful effects. It is difficult to measure the signal transduction effect of a plant under stress conditions. Heavy metal toxicity affected the plant physiological and biochemical process and reduces the growth and yield. Singh et al. [\(2016b](#page-21-1)) described the toxicity of metals and the plant responds in following ways:

- 1. Sensing of external stress stimuli.
- 2. Signal transduction and transmission of a signal information into the cell.
- 3. Triggering suitable precaution measures for counter the adverse effect.

Metal toxicity reduces the plant mitosis and root elongation process (Hossain et al. [2012a](#page-18-8), [b](#page-18-9); Thounaojam et al. [2012\)](#page-21-10). Some of the metals are analogs of plant essential nutrients, and plant uptake mechanism cannot identify the metal and reach into plant parts (Sivasankar et al. [2012](#page-21-9)). To avoid the toxicity, the plant having self mechanism and survive in contaminated soils in following ways:

16.3.1 Physical Barrier

A sophisticated and inter-related network of self defense mechanism in plant playing a vital role to avoid metal negative effect under stress. Physical barriers are the first line on defense mechanism, in which cell wall, trichomes, and various types of plantmicrobial associations are reducing the metal toxicity in plants (Hall [2002](#page-17-8); Wong et al. [2004;](#page-22-4) Harada et al. [2010](#page-17-9)). Most of the cases trichomes accumulated the heavy metal orproducing the many types of secondary metabolites to detoxification of metals in plants (Lee et al. 2002 ; Hauser 2014). If the metal pass through the physical barrier and reached to a cell site, than biosynthesis of different cellular biomolecules are acting as a potential heavy metal neutralizer. During the metal entry into the root, and transfer to the shoot part (mostly in hyperaccumulators) and avoid the metal toxicity by deposing metals in vacuoles (Fahr et al. [2013\)](#page-17-10).

16.3.2 Uptake by Hyperaccumulators

Hyperaccumulator are those plants having higher capacity of metal absorption without affecting growth activities (Ma et al. [2001](#page-19-4)). It has extraordinary capacity to absorb the metal ion concentration from contaminated sites (Yang et al. [2002\)](#page-22-1). Nowadays plant based metal removal is not in practice due to slow process and limited bioavailability of metals and greatly influenced by the climatic conditions of the regions (Mandal et al. [2016\)](#page-19-5). Hyperaccumulator plants are not accumulated higher amount of metal in different part due to novel genes, but due to differential expression of genes (Verbruggen et al. [2009\)](#page-21-11). A complete mechanism of heavy metal uptake by hyperaccumulator and non-hyperaccumulator are described in Fig. [16.1](#page-6-0). Most of the cases plant interact with the heavy metals and affected due to, (1) absorption of plant nutrient, for example, some of the heavy metals are analogs of essential plant nutrients As for P, and Cd for Zn; (2) direct interaction with functional protein groups, i.e. sulfhydryl group (-SH); (3) generation of reactive oxygen species (ROS), it damages the plant cell (DalCorso et al. [2013\)](#page-15-10). Sundaramoorthy et al. (2010) (2010) reported that Cr (VI) extended the cell cycle, and leads inhibitor effect on cell division and reduced the growth of the paddy plant. Later on, Yuan et al. [\(2013](#page-22-5)) evaluate the toxic effect of Cd and found that, it affected the cell elongation and meristem zones by modifying the auxin distribution via protein and reduce the primary root elongation process. Most of the cases toxic metals affected the functions of each other metals in harmful ways in biological systems.

16.3.3 Role of Metal Analog and Protein

Some of the metals having similar type of physico-chemical properties and plant could not identify the essential plant nutrients or competitive environment reduce the metal uptake by plants. Increasing the application of sulfur (S) reduced the uptake

Fig. 16.1 Mechanism of heavy metal uptake and defense mechanisms. (Adopted from Singh et al. [2016b](#page-21-1))

pattern of Se in Brassicaceae family plants (Galeas et al. [2007\)](#page-17-11). Increasing concentration of Zn reduces the uptake of Fe and Mn (Sivasankar et al. [2012\)](#page-21-9); Ni toxicity induces Zn deficiency in plants (Khan and Khan [2010\)](#page-18-7). Increasing concentration of Zn reduced the Cd uptake in T. caerulescens, due to control of ZIP gene on Zn (Assuncao et al. [2010](#page-14-3)). The heavy metal uptake from the soil and transported to plant parts via xylem with the help of various proteins, i.e. Heavy metal transporting ATPases (or CPx-type, P1B-type), Natural resistance-associated macrophage proteins (Nramp), Cation diffusion facilitator (CDF) family proteins, Zn–

Fepermease (ZIP) family proteins, and MATE (Multidrug And Toxin Efflux) protein family. All the proteins are having a specific role in metal tolerance by various plants. The CPx-type ATPases involved in transportation of metal such as Cu, Zn, Cd and Pb (Williams et al. [2000](#page-22-6)); whereas, P1B ATPases type protein regulates metal tolerance and homeostasis in hyperaccumulators (Axelsen and Palmgren [1998\)](#page-14-4). Similar type other protein like CDF involved in regulating cytoplasmic cation activities (Maser et al. [2001\)](#page-19-6).

16.3.4 Soil Enzyme Mechanism

Soil enzymes are the key factors for the determination of any anthropogenic disturbance in soil fertility. These are more sensitive to metal toxicity and affect the plant nutrient dynamics in soil. Soil enzymes make a temporary complex with metal and enhance the availability to plants (Segel [1975\)](#page-20-9). Most of the cases in the soil, enzyme inhibitor (reduce the reaction rate) and activators (increasing the reaction rate) are found and determined the level of soil enzymes. The effect of metals on enzyme functions are a complex mechanism and may also affected by soil (type of soil, pH, EC, organic matter, texture), plant (metal bioaccumulation, transfer ratio, plant type and cultivar), metal (type concentration, mode of action) and also by enzymatic properties (type and sensitivity, structural inhibition) (Tabatabai [1994](#page-21-13); Karaca et al. [2009,](#page-18-12) [2010](#page-18-13), [2011;](#page-18-14) Dotaniya et al. [2017d,](#page-17-4) [2018a](#page-17-12)). The soil enzyme reaction rate controlling by the inhibitor and activator is described in Fig. [16.2](#page-7-0) (Voet and Voet [1995\)](#page-21-14). Most of the cases, metal characteristics are playing a crucial role; however, Cd affected more negatively than Pb due to its greater mobility and lower affinity with soil colloids (Khan et al. [2007\)](#page-18-2). Another study conducted by Shen et al. [\(2005](#page-21-15)) found a negative correlation between Zn and Cd metals due to sorption and exchange sites in the soil.

16.4 Mechanism of Metal Tolerance in Microbes

Microbes are the scavenger of contaminants and reduce the metal toxicity in soil towards plants (Saha et al. [2017a\)](#page-20-0). It is responsible for the nutrient dynamics in soilplant system that affects the soil to soil solution and also solution to plant-roots movement/availability (Dotaniya et al. [2013c](#page-16-10), [2014b](#page-16-11), [e,](#page-16-12) [2015,](#page-16-13) [2016a](#page-16-0); Dotaniya and Datta [2014](#page-15-11)). These are also responsible for plant growth regulators/promoters secretion in plants under metal stress condition to protect them from the stress (Dotaniya et al. [2016a](#page-16-0)). Immobilization of nutrients and metals from soil by microbes is also widely accepted (Dotaniya et al. [2014f](#page-16-14), [2016c\)](#page-16-15). The researchers are isolated specific type of soil microorganism for the degradation of toxic metals. Microorganisms are omnipresent in nature and engage in nearly all biological processes of life (Singh et al. [2016a](#page-21-8)). Metal toxicity occurs in any ecosystem by either natural or anthropogenic enrichment or by both the means. Higher amount of metals in the environment is harmful to microbes, plant, animal and human. Due to increasing and enlarging area under urbanization and industrial activities, proportion of metals use tremendously increased nowadays and resulted in higher accumulation of metals in ecological habitats (Rajkumar et al. [2012\)](#page-20-10). Occupation of metals in native binding sites of microbial cells that is specifically for essential nutrients or metals and through ligand interactions result in metal toxicity in microbes (Bruins et al. [2000](#page-15-12)). For instances, Hg^{2+} , Cd^{2+} and Ag^{2+} are likely to bind with SH groups of some sensitive enzymes and hinter the function of the enzymes (Nies [1999\)](#page-19-7). But at higher concentration whether be a essential or non-essential metals can damage the membranes of microbial cell wall and interrupt the function of the cells by damaging DNA structure and altering enzyme specificity (Bruins et al. [2000\)](#page-15-12). However, some of heavy metal resistant microbes are adaptive to heavy metal rich environments. The possible mechanisms of metal resistance systems in microbes are identified and are elimination through permeability barrier; enzymatic reduction; capturing and sequestering in the cell (either intra- or extra-cellular means); active efflux pumps; and diminution in the sensitive cellular targets to metal ions (Bruins et al. [2000](#page-15-12); Nies [1999;](#page-19-7) Rensing et al. [1999](#page-20-11)). These mechanisms responsible for microorganisms to overcome metal toxicity and help them function well enough in contaminated environments (Dotaniya et al. [2018d\)](#page-17-13). The energy-dependent active efflux of toxic metal ions is mostly recognized in the largest group of metal resistance microbes. Further, many plasmids and chromosomal responsible metal tolerance mechanisms in bacteria have also been documented.

Biosorption of metals by the bacterial cells is mostly characterized by non-enzymatic process such as, adsorption. Increasing the amount of crop residue in the soil, provide the food material to soil biota that enhance the microbial population and their diversity as well as activity and improve nutrient bio-availability in the soil (Rajendiran et al. [2012;](#page-20-12) Dotaniya [2013;](#page-15-13) Dotaniya and Kushwah [2013](#page-15-14); Dotaniya et al. [2013b\)](#page-16-7). Mineralization and release of various types of C substrate during the decomposition of crop residue act as a biosorption for metals in soil (Kushwah et al. [2014;](#page-18-15) Prajapati et al. [2014,](#page-20-13) [2016\)](#page-20-14) and siliceous material also provide immunity to crop plant (Meena et al. [2013](#page-19-8)). Polysaccharides

and proteins associated with cell surface or extracellular surfaces are involved in adsorption of metals and this is a non-specific binding process (Rajkumar et al. [2010\)](#page-20-15). However, it can be, depending upon the microbial species, either active or passive process and/or both. In addition to chitosan and glucans, chitin present in the cell wall of microbes believed to be effective biosorbent. For instances, the cell walls of fungi, yeasts, and algae, are also reported to be an efficient metal biosorbents. Bioaccumulation of metals in microbial cells is an metabolic energy dependent active process (Martino et al. [2003](#page-19-9)). Potential metal bioaccumulation mechanisms in the bacterial cell membranes include carrier mediated transport, ion pumps and channels, complex permeation, endocytosis, and lipid permeation. These mechanisms are generally involved in transport of metals like Hg, Pb, Ag, Cd and Ni. The bacterial detoxification of arsenic is often carried out through the chemiosmotic gradient and the intracellular As concentration can be reduced by the active export mechanism through simple As^{3+} efflux systems (Rensing et al. [1999\)](#page-20-11). However this system is not involves in transport of As^{5+} . Therefore, As^{5+} is converted to As^{3+} by the arsenate reductase enzymes which enables microbes to detoxify both the As species. Similarly, Pb resistance also based on metal ion efflux system, i.e. through zinc and cadmium specific pumps in bacterial cells and also Pb-phosphate precipitation within the cells of metal tolerant bacterial species (Nies [1999;](#page-19-7) Rensing et al. [1999\)](#page-20-11). Microbial transformation of metals through oxidation and reduction and methylation and demethylation also considered as important resistance mechanisms in microbes. For example, microbes can acquire energy through oxidation of Fe, S, Mn and As (Santini et al. [2000\)](#page-20-16). On the other hand, microbes during anaerobic respiration can convert the metals into its reduced state/ form through dissimilatory reduction. With this process metal can act as a terminal electron acceptor. Oxyanions of As, Cr, Se and U are the terminal electron acceptors used by the microbes during anaerobic respiration process (Turpeinen et al. [2002\)](#page-21-16). Moreover, reduction process performed by the microbes is not mainly linked to respiration, but to impart metal resistance. Aerobic and anaerobic reduction of Cr^{6+} to Cr^{3+} , Se⁶⁺to Se⁰, U⁶⁺to U⁴⁺ and Hg²⁺to Hg⁰ are generally carried out by the microbes to detoxify them.

Biomethylation of metals becomes resulted in formation of volatile compound of metal. In case of mercury, Hg(II) can be transformed into methylmercury by different group of bacterial species (e.g. Bacillus sp., Clostridium sp., Escherichia sp. and Pseudomonas sp.) and methyl mercury is volatile in nature, easily absorbed and accumulated an also highly toxic Hg species. In the same way, As is transformed into arsines, selenium is converted to to dimethyl selenide and Pb to dimethyl Pb (Gao and Burau [1997](#page-17-14); Pongratz and Heumann [1999](#page-20-17); Dungan and Frankenberger [2000\)](#page-17-15). In addition to above phenomenons, high concentrations of As, Cd, Cu, Co, Ni and Zn are leached out from contaminated areas by acidophilic iron- and sulfuroxidizing bacteria (Groudev et al. 2001). Moreover, sulfate-reducing bacteria, on the other hand, can precipitate metals into sparingly soluble metal sulfide compounds through metabolic processes (Lloyd and Lovely [2001](#page-18-16)). In another study, the resistance against copper by Pseudomonas syringe has been reported and it is mainly due to the Cu accrual and compartmentalization in the cell's outer membrane and the

periplasm (Cooksey [1993\)](#page-15-15). Either microbs can increase the bioavailability of metals by solubilizing and mobilizing the insoluble metals become potentially toxic or reduce their bioavailability by immobilization processes. These kinds of bio-transformation processes of metals are major components of the metals biogeochemical cycles to maintain the proper ecosystem functioning. The microbes involved in metal decontamination processes can be further exploited in remediation of contaminated environments.

16.5 Microbe-Plant-Metal Interaction

Plants and microbes are generally coexisting in nature at any given ecosystems and they may symbiotic or compete with one another for their survival. Microbes and plant root exudates play a major role in functioning of rhizosphere ecology and influence the bioavailability of metals and nutrients in rhizosphere soil. Microbes help in stimulating plant root exudation and the root exudates are generally rich in carbon can be used as food and energy sources by microbes. Cohesive plant-microbe associations can play a very important role in adapting to metal rich environments can be focused further to advance microbe-mediated phyto-remediation. The metal mobility and availability can be influenced and phytoremediation efficiency of plant enhanced by root exudates through (1) proton $(H⁺)$ release 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 of rhizosphere soil through enzyme mediated e^- transfer and (4) enhanced microbial activity in the rhizosphere (Sessitsch et al. [2013\)](#page-21-17). In this connection, Kim et al. ([2010\)](#page-18-17) have reported that translocation and bioaccumulation of metals are significantly enhanced by citric and oxalic acid and suggesting that these acids can be used as natural chelating agents for better phytoextraction. Further, microorganisms particularly growth promoters (PGPMs) like some beneficial fungi and bacteria can involve in reducing phytotoxicity of metal by indirectly improving plant growth through stimulating defence mechanisms in opposition to phytopathogens and directly through generation of growth promoting substances, enzyme secretion and mineral nutrients solubilization of (N, P, K, Fe, etc.). Microbes induce or enhance phytoremediation of plant by improving its biomass growth and influencing metal availability and facilitate for bioaccumulation from soil -root and translocation from root-shoot (Ma et al. [2013](#page-19-10)).

To recruit the beneficial microorganisms and to make better plant-microbe interrelationship plant roots selectively exudates plant metabolites (organic compounds) that are effectively signals bacteria and fungi for its association. Each plant species has its characteristic group of associated microbes and able to link up with them by selection from surrounding soil environments for creating its own root microflora (Hartmann et al. [2009](#page-18-18)). This mechanism is directly associated with the type and amount of root exudates produced as well as rhizosphere soil features. In rhizospheric zone, microbes can establish proficient symbiosis with plants through triggering host functional signals (chemotaxis and colonization) and plants can well

communicate with their adjoining soil microorganisms by signals or root exudates (Bulgarelli et al. [2013](#page-15-16)). Besides chemotaxis, rhizo-bacterial colonization can be initiated by electric gradients (electrotaxis) created by plant roots believed to be a possible mechanism (Lugtenberg and Kamilova [2009](#page-18-19)).

Presently, results from number of studies have revealed that beneficial microbes mediate plants to acquire sufficient mineral nutrients (such as N, Ca, Fe, Mg, and P) in metal contaminated soils, thus, establishment of highly developed and thriving root system in the initial crop growth stages is highly advantageous in phytoremediation of metal polluted soils (Ahemad and Kibret [2014](#page-14-5)). For instance, N fixing bacterial groups like Rhizobium, free living bacterial species in rhizosphere and endophytic bacteria can improve the soil fertility polluted areas resulted in enhancing plant growth and N concentration in roots and shoots (Wani et al. [2007\)](#page-21-18). Similarly, in zinc/lead mine tailings arbascular mycorrhizal fungi that absorb and mobilize nutrients helped plant growth and nutrient uptake by leguminous trees grown on the contaminated tailings (Harris and Lottermoser [2006](#page-17-17)). Moreover, microbes transform highly insoluble metal sulphides to readily available form that enables the hyper-accumulator plants to remove toxic metals from soil solution. This will provide additional attractive options for microbes to improve metal resistance ability per se (Sharma et al. [2000](#page-21-19)). Furthermore, siderophores and H^+ are specifically generated by soil microorganisms under iron (Fe) deficiency conditions. Of late the role of siderophore producing microbes (SPMs) such as bacteria and fungi involved in Fe acquisition of different plant species and related mechanisms behind their promotion of Fe acquisition has been extensively studied (Gaonkar and Bhosle [2013\)](#page-17-18). Even under stress conditions, phytohormones produced by plant associated microbes such as IAA, cytokinins, GA, ABA and others, can govern the hormonal balance in plants as a response to stress (Ullah et al. [2015;](#page-21-20) Ma et al. [2016](#page-19-11)). Also, Arbascular mycorrhizal fungi colonization in the plant root zones also has constructive effects on plant cell growth and division because of fungal hormones production (Yao et al. [2005\)](#page-22-7). The alteration in endogenous phytohormones levels are also accountable for morphological changes encouraged by AMF inoculation.

Apart from the above beneficial mechanisms, soil microbes involve in initiation of synthesis of ethylene inhibitors to support plant growth under stress conditions, (Glick [2014](#page-17-19)), antimicrobial enzymes (Saima et al. [2013](#page-20-18)) and polysaccharides (Naseem and Bano [2014\)](#page-19-12). These play a major role and enable plants to overcome or copeup with the negative impact of both biotic (fungi or harmful insects) and abiotic stresses (such as waterlogging, drought, salt stress, and metals toxicity; Fig. [16.3\)](#page-12-0). Production of ACC deaminase by plant growth promoting bacteria is one among the key traits which hydrolyses ACC, plant ethylene precursor, to $NH₃$ and ketobutrate (Glick [2014](#page-17-19)). In spite of above, plant growth and biomass improvement through root modification by inoculating efficient fungal and bacterial species under compatible environment of plant-microbe-site combinations. This can be envisaged through advanced biotechnological applications in phytoremediation. In general, plant associated microorganisms can promote plant growth and development by resorting to any one or more of the above mechanisms. For that reason, PGPM can be effective utilized in stressful environments for phytoremediation of

Fig. 16.3 Pictorial outline of the plant-microbe-metal interactions for heavy metal decontamination of polluted soils

metals. Apart from inherent capabilities, the usefulness of PGPM for higher plant growth is mainly associated with intimate interaction with host plant and soil characteristics. However, in future, contribution of genes in relation to phytobeneficial traits and occurrence of preferential symbiosis needs to be studied in-depth to harness the benefit of plant-microbe interactions.

16.6 Can Heavy Metal Uptake Mediated by Climate Change?

Climate change effect on crop productivity and water use efficiency are clearly observed by the various researchers (Amrawat et al. [2013;](#page-14-6) Jajoria et al. [2014;](#page-18-20) Meena et al. [2016](#page-19-13), [2017b\)](#page-19-14). In major crops, increasing temperature enhances the respiration rate and reduces the crop yield (Dotaniya [2015](#page-15-17); Dotaniya et al. [2018b\)](#page-17-20). This situation is more pathetic in tropical and sub-tropical countries like India, Sri Lanka etc. (Kundu et al. [2013;](#page-18-21) Meena and Dotaniya [2017](#page-19-15)). Increasing the greenhouse gases concentration in the atmosphere and elevated the temperature due to more absorbance of shortwave radiations, generate global warming effect (Dotaniya et al. [2017b](#page-16-16); Meena et al. [2017a](#page-19-16)). Increasing the temperature slightly enhanced the photosynthesis activity in temperate regions where temperature acts as a limiting factor (Bharti et al. [2017](#page-15-18)). Direct effects of climate change on heavy metal uptake by the plant are even sparser (Wijngaard et al. [2017\)](#page-22-8). Heavy metal update pattern indirectly affected by the climate change effect. Increasing the root exudates as low molecular organic acids mediated the availability of heavy metals in soil (Dotaniya et al. [2016b\)](#page-16-17). However, increasing the root exudates enhance the microbial count and diversity in the soil. Increasing the microbe's population accelerates the decontamination process in the soil and reduces the toxicity. The soil enzyme activities in soil, increase and decrease by the metal type, speciation, availability and toxicity (Shen et al. [2005;](#page-21-15) Yang et al. [2006](#page-22-2); Khan et al. [2007;](#page-18-2) Karaca et al. [2010\)](#page-18-13). Extracellular enzyme like phosphatases and dehydrogenase enzyme activities accelerated the decomposition of organic matter (Bell et al. [2010;](#page-14-7) Dotaniya [2015](#page-15-17)) and enhanced the metal availability in soil solution or appear toxicity in plants. Dhillon et al. $(1996a, b)$ $(1996a, b)$ $(1996a, b)$ $(1996a, b)$ reported that under elevated $CO₂$ concentration extracellular enzymatic activities increased due to microbial demand for N and P. Rate of microbial immobilization can also increase (Mikan et al. [2000](#page-19-17)) or decrease (Berntson and Bazzaz [1997](#page-14-8)) with elevated CO_2 concentration. Increasing global precipitation accelerated the metal mobility via biogeochemical cycles and also enhances the metal availability and uptake by plants (Carillo-Gonzáles et al. [2006;](#page-15-21) Reeder et al. [2006\)](#page-20-19). These processes also accumulate heavy metals in soil and sediment, due to sorption mechanisms (Foster and Charlesworth [1996\)](#page-17-21). Increasing the precipitation rate can dissolve the heavy metals from contaminated area and transported into a new region; it acts as a base for metal uptake. Larger amount of heavy metals moved from various parts of contaminated sites to uncontaminated areas and accumulate in the upper soil furrow (Rozemeijer and Broers [2007;](#page-20-20) Bonten et al. [2012](#page-15-22)). Use of marginal quality water is an alternative water management strategy for combating the adverse effect of climate change. Increasing wastewater use in water scarceareas for the cultivation of crops in the developing countries is more prone to contamination of toxic metals via food chain contamination (Meena et al. [2015](#page-19-18)). The repeated irrigation with poor quality water accumulated more amounts of heavy metals in soil and enhances the metal uptake by plants.

16.7 Future Line of Research

- Effect of climate change on heavy metal dynamics with respect to plant metabolites.
- The interaction effect of various metals on each other dynamics and its toxicity effect on soil microbial population.
- Genetic engineering assisted phytoremedition and its effect on root exudations.
- Plant metal uptake pattern in various crops with respect to water and soil conditions.
- Soil organic carbon dynamics and its effect with root exudates on metal dynamics.

16.8 Conclusions

Soil water pollution is the challenging task to remediate for the sustainable agricultural crop production. Increasing population forced per hectare more food grain, which is limited by the potential capacity of natural resources. Increasing use of poor quality natural resources, enhance the metal toxicity in human beings via food chain contamination. Most of the peri-urban areas of metropolitan cities are using industrial or sewage water for cultivation of crops specially vegetable production. Repeated application of metal contaminated effluent for agriculture purpose accumulated huge amount of heavy metal in the field. The Bioremediation is a one of the low cost technology for the heavy metal remediation. In which, bio-agent (plant or microbe) are using for minimizing of metal toxicity from the soil and plant environments. Microbes secreted various types of organic acids, reduced or convert toxic metal to non toxic. However, it improved the soil physico-chemical properties and increases the crop sustainability in contaminated soils. Use microbial assisted phytoremediation can reduce the contamination level in soil; it is a low cost, eco-friendly and more viable than phytoremediation techniques.

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