Chapter 14
Growth Enhancement and Bioremediation of Heavy Metal in Crop Plants Through *Bacillus* Species Application

Saidu Abdullahi, M. A. Baset Mia, and Amir Hamzah Ghazali

Abstract Mitigating the effects of heavy metals and their subsequent remediation on plants is one of the hot topics of environmental research studies. Application of *Bacillus* species in this area of research has received considerable attention probably due to the high rate of adaptability and survivability of the species under extreme environments. *Bacillus* spp. have shown great potential in plant growth enhancement and bioremediation of heavy metal–contaminated soils. More *Bacillus*–plant physiological studies are required for better understanding of the mitigation mechanisms of *Bacillus* spp. against heavy metal stress conditions and plant growth promotion. Our findings have successfully shown that Bacilli have multiple beneficial traits which assisted the crop plants either directly or indirectly through plant growth–promoting activities and heavy metal tolerance enhancements.

Keywords Bioremediation · Heavy metal toxicity · Growth enhancement · Crop plants · Environmental pollution

S. Abdullahi
School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia

Department of Botany, Ahmadu Bello University, Zaria, Nigeria

M. A. B. Mia (✉)
Department of Crop Botany, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh

A. H. Ghazali (✉)
School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia
e-mail: amirhg@usm.my

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14.1 Introduction

Bacillus species represents one of the most important and dominant groups of microbes that exist in various environments with a high rate of survival in extreme and adverse environmental conditions (Islam et al. 2019). The gram-positive or facultative gram-negative spore-forming bacteria can survive for a long time under unfavourable environmental conditions (Radhakrishnan et al. 2017), and spore formation is one of the most important characteristics of *Bacillus* spp. The aerobic endospore-forming bacteria are ubiquitous in agricultural systems due to possession of certain physiological traits associated with their survival which include production of a multilayered cell wall, production of endospores that are stress-resistant, secretion of antibiotics, peptide signal molecules, and extracellular enzymes (Gardener 2004). The cell-wall-degrading substances (protease, cellulase, chitinase, glucanase, hydrogen cyanide, and lipopeptides) from *Bacillus* spp. damage the pathogenic organisms including bacteria, fungi, viruses, nematodes, and pests to control their populations (Radhakrishnan et al. 2017). *Bacillus* spp. also have great potential for applications in agriculture, industry, and medicine and are a good source of important metabolites and enzymes of various biotechnological interests. As an example, it was shown that *B. thuringiensis* H-14 is able to kill mosquito larvae within 24 h of treatment with the lowest concentration of spore-crystal suspension (0.05 mg/L) (Fun et al. 2016).

There is an increasing interest in *Bacillus* spp. on the aspects of agricultural and environmental biotechnology due to increasing demand for food production, recovery of degraded soils as well as the development of biofertilizers and biopesticides (Nascimento et al. 2020). The unfavourable biotic and abiotic stimuli affect normal plant metabolism, suppressing the growth and yield of plants, however, the stress factors on crops are mitigated by *Bacillus*-induced physiological changes (Radhakrishnan et al. 2017). Under unfavourable environmental conditions including heavy metal stress, *Bacillus* produces important substances such as siderophores and exopolysaccharides, which prevent the movement of toxic ions and adjust the ionic balance and water transport in plant tissues (Radhakrishnan et al. 2017). *Bacillus* can live both outside and within plant tissues, facilitating plant growth and development as well as protection from harsh environmental conditions through several mechanisms including N_2 fixation, production of indole acetic acid (IAA), siderophore, and 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Additionally, phosphate and potassium solubilization and production of exopolysaccharides show antagonistic actions against pathogens and other pests. There are many bacterial species that are being applied as plant growth-promoting bacteria (PGPB) to crop plants; however, members of the *Bacillus* group are more favoured for commercialization as PGPB due to some features including their ability to produce the heat and desiccation-tolerant endospores, which help to maintain high cell viability and prolong the shelf life of the bacteria in the carrier formulations (Akinrinlola et al. 2018). Bacterial colonization of roots provides a nutrient source, and the plants on the other hand, receive bacterial metabolites and other stimuli that enhance growth and stress resistance (Hashem et al. 2019). In this relationship, the microsymbiont (e.g. *B. subtilis*) will form a thin biofilm layer on the root surfaces for long-term bacterial colonization of the host plants (Hashem et al. 2019). Additionally, the rhizosphere of the host plants is also heavily colonized by beneficial bacterial populations. Nowadays, plant growth-promoting *Bacillus* spp. are gaining prominence as biofertilizer, biopesticides, and bioremediator of certain mineral elements that are toxic to crop plants (Amir et al. 2005; Fun et al. 2016; Mia et al. 2010; Salwani et al. 2012; Tang et al. 2020). Salwani et al. (2012) revealed that the beneficial diazotrophic microsymbionts of leguminous cover crop *Mucuna bracteata* were not only from the conventional Alphaproteobacteria class (e.g. *Brevundimonas* sp.) but were also from the Betaproteobacteria class (e.g. *Achromobacter* sp. and *Burkholderia* sp.) and the Gammaproteobacteria class (e.g. *Stenotrophomonas* sp.). The authors also successfully obtained and identified *Bacillus* sp. from the root nodules of the host plants.

Heavy metal may be found naturally in the soils or added to the soils through various anthropogenic activities such as exploitation of mines and smelters, fossil fuel combustion, metallurgical and electronics industries, indiscriminate waste disposal, and military training. Additionally, the agricultural practices involving excessive application of inorganic fertilizers and chemicals for optimum crop improvement are also contributing towards the escalated accumulation of heavy metal in soils (Kapahi and Sachdeva 2019; Oves et al. 2016). Examples of heavy metals which are contaminating soils and water bodies include As, Cd, Pb, Hg, Cr, Co, Cu, Hg, Ni, Zn, Se, U, Mn, and Ni (Weissmannová et al. 2019). The continuous release of heavy metals into the environment severely affects soil and water quality. Despite the fact that waste water may serve as an important source of essential nutrients for plants, many risks can be attributed to the use of such waste water for crop irrigation due to presence of toxic contaminants including heavy metal (Khalid et al. 2018). Many heavy metal are toxic even at very low concentrations, and some (e.g. As, Cd, Cr, Hg, Ni, Cu, Pb, and Zn) are not only cytotoxic but also mutagenic and carcinogenic in their nature (Dixit et al. 2015).

Heavy metal contaminated soils are generally deficient in essential nutrients, and if the contaminated soils are used for crop production, there is a huge risk of metal being transferred into food chain at higher concentration. It can cause severe human health problems (Oves et al. 2016). Certain heavy metal is essential for plant growth and health at certain concentrations. For example, Co is required in nitrogenase enzyme as metal activator for N_2 fixation both in legume and non-legume crops, and Mo is needed as catalytic centre of many enzymes namely nitrate reductase. However, when the elements exceed the required quantities, they may cause serious injury or even lead to death of plants (Oves et al. 2016). Biological methods for remediation of heavy metal known as bioremediation are considered most eco-friendly, cost-effective, reliable, and have no adverse effects to the environment (Dixit et al. 2015). There is an increasing interest towards studying the role of microorganisms in biotransformation and detoxification of heavy metals and the production of plant growth promoting substances by them under stress conditions (El-Meihy et al. 2019; Nayak et al. 2018). Understanding various mechanisms of metal accumulation and plant growth promotion has numerous biotechnological implications for bioremediation of heavy metal and crop productions in heavy metal contaminated soils. Mitigating the effects of heavy metal and their subsequent remediation on plants is one of the hot topics of environmental research nowadays. Application of *Bacillus* species in this area of research has received considerable attention (El-Meihy et al. 2019; Li et al. 2019; Tang et al. 2020) probably due to the high rate of adaptability and survivability of the species under extreme environments (e.g. heavy metal stress tolerance and their multiple beneficial mechanisms of actions) (Radhakrishnan et al. 2017). However, very few study reports are available on the application of *Bacillus* in bioremediation of heavy metal in plants. Most of the relevant studies were carried out on the physiological mechanisms of plant growth promotion or heavy metal tolerance of the individual *Bacillus* spp.. Rhizobacteria viz. *Bacillus* spp. (*B. megaterium, B. cereus,* and *B. pumilus*), isolated from *Ludwigia octovalvis,* were capable of absorbing As and can be good candidates for bioremediation of toxic effects of that element on plants (Titah et al. 2018). More studies on heavy metal tolerant and plant growth promoting *Bacillus* and other bacteria to harness their potentials as bioremediation or phytoremediation agents are therefore still needed. In this chapter, potentials of *Bacillus* spp. on promoting growth of associated selected host plants and their ability to remediate heavy metal are discussed.

14.2 Production of Plant Growth Promoting (PGP) Substances by *Bacillus* Species

14.2.1 Production of PGP Substances by Individual Bacillus *Species Under Culture Conditions Supplemented with Heavy Metal*

Some of *Bacillus* spp. namely *B. sphaericus* strain UPMB10 are able to produce phytohormone like auxin and gibberellin which resulted in increased root growth in banana and oil palm under hydroponics and pot culture conditions (Mia et al. 2010; Amir et al. 2005). A large body of studies reflected that *Bacillus* species can produce plant growth promoting substances even under heavy metal stress conditions (Wu et al. 2019). Rizvi et al. (2019) also stated that heavy metal tolerant *B. subtilis* strain BM2 was able to synthesise variable concentrations of indole acetic acid (IAA) when cultured under different concentrations of Pb and Ni. However, the highest concentration of IAA was produced by the bacterial strain (BM2) when cultured in metal free medium (51.6 μg/mL), which kept declining with increasing concentrations of the added metal. At 400 μg/mL Pb and Ni, IAA secretion was reduced by 58 and 47%, respectively. Despite the toxicity and inhibitory effects of the metal, the strain maintained reasonable amount of IAA production potentiality. The strain was also able to produce substantial amount of ACC deaminase and siderophore when cultured in media supplemented with high concentrations of metal. However, the siderophore production by the strain was not detected on CAS agar with metal supplementation but detected under liquid medium with the metal supplementation. The cause of siderophore not detected in CAS agar but in liquid medium with metal supplementation remains unknown, but likely due to the presence of HDTMA (hexadecyltrimethyl-ammonium bromide) in the solid agar (Rizvi et al. 2019).

Bacterial IAA induce root elongation and development to overcome heavy metal toxicity and improve plant growth, while ACC deaminase, on the other hand, metabolizes the ethylene precursor ACC, thereby alleviating the effects of ethylene stress due to the metal and helps in plant survival under stress conditions (Mishra et al. 2017). Siderophore produced by *Bacillus* and other plant growth promoting bacteria is one the most important substances that can directly alleviate heavy metal toxicity by binding/chelating heavy metal ions and increasing the supply of iron to plants (Nayak et al. 2018). Therefore, maintenance in the production of plant growth promoting substances by *Bacillus* spp. under heavy metal stress could play an important role in improving growth of the host plants in addition to reduce the toxic effects of the metal on the plant.

14.2.2 Production of PGP Substances by **Bacillus** *Species in Association with Plants*

Nitrogen is an important essential nutrient which is required for growth and development of plants, taken up as NO_3^-, NH_4^+, and N_2 (*via* biological nitrogen fixation process) (Bellogín et al. 2014). Atmospheric nitrogen fixing bacteria form symbiotic association on the roots of plants where they convert nitrogen into form that can be used by plants, a process mainly restricted to leguminous plants. However, several non-symbiotic bacteria have been recognized including many *Bacillus* spp. as free living nitrogen fixers in many plants of agronomic importance. Various species of *Bacillus* were reported to fix atmospheric nitrogen which include *B. cereus, B. marisflavi, B. megaterium, Paenibacillus polymyxa,* and *P. massiliensis* (Ding et al. 2005; Halim et al. 2016; Nayak et al. 2018; Tang et al. 2020). Others were able to solubilize phosphates like *B. cereus, B. thuringiensis, B. megaterium, B. safensis, P. cineris,* and *B. subtilis* (Akinrinlola et al. 2018; Babu et al. 2013; Huang et al. 2020; Zaidi et al. 2006). Phosphorus, which mostly occurs in the soil in the form of non-soluble compounds and thus not always available for plant consumption (Bellogín et al. 2014), is converted to soluble form that can be absorbed and utilized by plants in the process of phosphate solubilization. Besides that, PGPB inoculation can also stimulate and increase uptake of other nutrients such as K, Ca, Fe, Cu, and Zn which may be achieved through acidification of the rhizosphere (e.g. by production of organic acids), and in general, decrease in soil pH increases solubilization of nutrients (Bellogín et al. 2014). Some *Bacilli* have iron-chelating properties through the production of siderophores that are known to solubilize iron in the rhizosphere from minerals and organic compounds (Radhakrishnan et al. 2017). The siderophores produced bind insoluble Fe^{3+} and reduce it to soluble Fe^{2+}, which can then be utilized by plants.

14.2.3 Role of Phytohormone (IAA) and Enzyme (ACC Deaminase) in Mitigating the Heavy Metal Stress in Plants

The IAA production by plant-associated bacteria plays an important role in plant-bacterial interactions. IAA synthesized by beneficial bacteria can increase the number of root hairs and lateral roots as well as the total root surface area, leading to an enhancement of root exudation and mineral uptake from the soil (Kong and Glick 2017). Number of studies have also suggested that PGPB producing IAA may play essential role in improving metal phytoremediation, however, most did not provide definitive proof of the direct involvement of the IAA (Kong and Glick 2017). In a previous study, two PGPB strains *B. paralicheniformis* YSP151 and *Brevibacterium frigoritolerans* YSP40 isolated from *Brassica juncea* in a Pb-contaminated mine soils were used to inoculate similar host plants grown in a metal-contaminated soil (Yahaghi et al. 2018). Results from the trial showed enhanced growth and Pb uptake by the inoculated host plants and both bacterial strains were reported to have high IAA producing ability. Multiple plant growth promoting traits may together be responsible for survival and enhanced metal tolerance of the host plants grown in heavy metal contaminated soils. Some bacteria can stimulate plant growth either by synthesizing more IAA or by degrading excess synthesized IAA when it is detrimentally higher than normal levels (Kong and Glick 2017).

Increase in ethylene synthesis is mostly associated with various environmental stresses such as heavy metal stress, extreme temperatures, drought, high salinity, organic pollution, insect damage, radiation, wounding, and various pathogens including viruses, bacteria, and fungi. Much of the plant growth inhibition that occurs due to environmental stresses is the result of increased levels of stress induced ethylene, which affects the plant's response to the stressor (Kong and Glick 2017). Some PGPB have great potential to decrease ethylene production that in turn enhance plant growth. This potential is due to the production of enzyme ACC (1-Aminocyclopropane-1-Carboxylate) deaminase. ACC is the precursor to ethylene synthesis and ACC deaminase enzyme hydrolyses ACC into a-ketobutyrate and ammonia (Ashraf et al. 2017). The ammonia generated add to the nitrogen source of the plants. ACC deaminase producing PGPB can facilitate plant growth and development by converting the ACC into α-ketobutyrate and ammonia, thereby reducing the levels of plant ethylene and providing some protection against growth inhibition caused by heavy metal and other stress factors (Kong and Glick 2017).

14.3 *Bacillus* Species and Heavy Metal Transformation, Detoxification, and Mobilization

Bacteria are known to enhance plant growth and survival under heavy metal stress conditions due to their ability of consuming and converting complex waste into simple non-toxic products/compounds (Tiwari and Lata 2018). Some heavy metal tolerant bacteria are also involved in enzymatic oxidation or reduction of toxic metal to a non-toxic or less toxic forms. Application of *Bacillus* spp. into heavy metal-contaminated soils can reduce the deleterious and toxic effects of heavy metal on growth of crop plants. For example, the Cr and As resistant *B. firmus* strain TE7 isolated from tannery effluent was able to reduce Cr(VI) to Cr(III) and oxidize As(III) to As(V) (Bachate et al. 2013). It reduced 100 mg/L Cr(VI) and oxidized 150 mg/L As(III) within 60 hours in nutrient broth and 10 hours in minimal medium. The hexavalent chromium [Cr(VI)] is the most toxic form of chromium, and arsenite [As(III)] the most toxic form of arsenic, and thus, their respective reduction and oxidation have great environmental importance as they affect their toxicity and mobility (Bachate et al. 2013). Furthermore, two *B. cereus* strains (*B. cereus* D and 332) were recently shown to have high Cr tolerance and reduction ability, and *B. cereus* D achieved 87.8% Cr(VI) removal in 24 h (Li et al. 2020). According to the authors, Cu (II) significantly increased the removal rate of the Cr (VI). Inoculation of *B. cereus* WSE01 was reported to increase the level of leaf enzymatic activity in *Myriophyllum verticillatum*, which, on the other hand, mitigate the effects of oxidative damage caused by reactive oxygen species resulting from heavy metal stress. Li et al. (2019) found that metal-resistant *B. thuringiensis* HC-2 was able to reduce the concentrations of Cd and Pb by extracellular adsorption and increasing the pH of the solution. Treatments of metal contaminated substrates with *B. thuringiensis* HC-2, biochar, and biochar combined with *B. thuringiensis* HC-2 significantly reduced water-soluble Cd and Pb concentrations by 34–56% and 31–54%, respectively and also increased the pH and NH_4^+ concentration in solution, in comparison to the values in a control.

14.4 Physiological and Biochemical Mechanisms of Heavy Metal Tolerance of *Bacillus*

The PGPB may contribute in reducing metal phytotoxicity through biosorption and bioaccumulation (Ma et al. 2016). Metal biosorption by bacteria comprised of two steps, which are passive and active biosorption (Ma et al. 2016). Passive biosorption of metal usually by living and dead or inactive cells take place in the cell wall due to number of metabolism-independent processes. Here, metal ions are adsorbed rapidly to the cell surface by reactions between metal and functional groups on the cell surface. Metal binding mechanisms like ion exchange, complexation, coordination, sorption, chelation, and precipitation may be involved. Active biosorption (bioaccumulation), on the other hand, is referred to the uptake of metal by living cells through a much slower active metabolism-dependent transport of metal into bacterial cells. In heavy metal detoxification, studies carried out using different species, *B. licheniformis* NSPA5, *B. cereus* NSPA8, and *B. subtilis* NSPA13, indicated a significant level of Pb biosorption, with *B. cereus* having the maximum of 87–90% (Syed and Chinthala 2015). The negatively charged functional groups such as hydroxyl groups, phosphate groups, carbonyl groups, etc. that are present in biomolecules of microbial cell wall surfaces bind readily to heavy metal ions (Ojuederie and Babalola 2017). One of the most essential constituent in bacterial cells having ion sequestration capability is exopolysaccharide (EPS) (Ojuederie and Babalola 2017) mainly composed of complex high molecular weight organic macromolecules. Exopolysaccharides are known to protect bacteria against environmental stresses such as heavy metal toxicity.

Other abiotic and biotic stress factors with adverse effects on growth and survival of crop plants are mitigated by *Bacillus*-induced physiological changes, which include the activation of antioxidant and defence systems (Huang et al. 2020), regulation of water transport, nutrient uptake, and enhancement of photosynthetic pigments (Babu et al. 2013), leading to increased crop tolerance and productivity.

14.5 Molecular and Genetic Basis for Plant Growth Promotion and Heavy Metal Tolerance Ability of *Bacillus* Species

Bacillus association triggers plant resistance and immunity against different stresses (including heavy metal stress) by altering stress-responsive genes, proteins, phytohormones, and related metabolites (Radhakrishnan et al. 2017). Studies on genomic analysis of species of *Bacillus* have revealed rich genetic elements involved in important plant growth promoting and heavy metal tolerance activities. For example, the genome of *B. megaterium* STB1 possess genes related to rhizosphere colonization, xenobiotic degradation, pathogen antagonistic activities and several other genes for multiple stress resistance (Nascimento et al. 2020). Genes associated with different plant growth promoting activities are found in many species of *Bacillus* under heavy metal stress. Ding et al. (2005) selected 29 isolates in a study aimed at identifying the possible nitrogen-fixing Bacilli from plant rhizospheres based on their ability to grow on nitrogen-free medium, out of which seven had *nifH* gene belonging to *Bacillus* and *Paenibacillus* genera. It was the first report of nitrogen fixation in *B. marisflavi* and *P. massiliensis* and the first of the *nifH* gene from *B. megaterium* and *B. cereus*. Also, the whole genome of *B. aryabhattai* AB211 was sequenced, with main focus on genomic elements related to plant microbe interaction (Bhattacharyya et al. 2017). Genome comparisons between the strain AB211 and other related strains of *B. aryabhattai* revealed about 3,558 genes which were conserved among all the genomes, with most genes involved in plant growth promotion activities found within core genes of all the genomes used for comparison. The findings showed possible common plant growth promoting traits shared among the strains of *B. aryabhattai*. Functional annotation of genes in the *B. aryabhattai* strain AB211 revealed the presence of many PGP genes which include those responsible for phosphate solubilization, siderophore production, exopolysaccharides production, and IAA production, most of which were experimentally verified in the study. Earlier findings by Halim et al. (2016) also proven that *Paenibacillus durus* ATCC 35681 is a free-living nitrogen-fixing bacterium, and the complete genome of the strain was successfully sequenced too. Interestingly, the isolate can also be found in a symbiotic relationship with plant roots.

Similarly, genes for the resistance, mobilization, detoxification, or transformation were detected in many *Bacillus* species. For example, genomic analysis of *B. megaterium* STB1 revealed abundance of genes for heavy metal resistance and transport, which include those encoding arsenate (*arsC*) and chromate (*chrR*) reductases and several others for Zn, Cd, Cu, Co, Mn, Ni, Cr, and As (Nascimento et al. 2020). One of the arsenate reductase genes in the genome occur in a cluster that contain other arsenate transport and resistance related genes. BLASTn analysis showed that the cluster is rare, and that close homologs were only found in *B. weihaiensis* Alg07 chromosome and *B. oceanisediminis* 2691 pB01. These bring in new insights into the capabilities and roles of *Bacillus* spp. as potential and important plant growth promoting bacteria with ability of not only improving plant growth but also enhancing the tolerance and survivability of the host plants under heavy metal stress conditions.

14.6 Enhancement of Plant Growth and Heavy Metal Tolerance of Plants in Association with Different Species of *Bacillus*

Representatives of plant-beneficial bacteria are widely spread among gram-negative and gram-positive bacteria, with *Pseudomonas* and *Bacillus* attracting main attention (Borriss 2014; Radhakrishnan et al. 2017). Despite the great progress in *Pseudomonas* research, its commercial use in agriculture is unfortunately limited due to difficulties in preparing stable and long-living bioformulations compared to *Bacillus* (Borriss 2014). Bacilli are increasingly and interestingly used commercially in agriculture to enhance yield of crops and reduce use of harmful agrochemicals (Ahmad and Saghir 2010; El-Meihy et al. 2019; Minaxi et al., 2012). When the plant growth promoting attributes of *Bacillus* sp. RM-2 were tested in both laboratory and field conditions, the isolate significantly increased growth ($P<0.05$) and yield parameters and nutrients uptake of cowpea plants (Minaxi et al., 2012). Metal tolerant *B. subtilis* BM2 was able to mediate the phytotoxic impact and significantly reduced Ni and Pb uptake in winter wheat and also improved plant growth (Rizvi et al. 2019). The BM2 strain was able to increase the grain yield significantly by

49% and 50% under 870 mg/kg Ni and 585 mg/kg Pb, respectively. The exceptional ability of the strain to produce plant growth promoting substances like siderophore, IAA, ammonia, and ACC deaminase under the metal stress condition might have played important role in achieving the overall performance and enabling continuous growth and survival of wheat even under the metal stress condition (Rizvi et al. 2019).

The *Bacillus* spp. are as well employed in the field of phytoremediation aimed at improving the uptake of heavy metal by hyperaccumulator plants (Babu et al. 2013; Ndeddy Aka and Babalola 2016; Tang et al. 2020). Recently, *B. cereus* strain WSE01, that can tolerate up to 1500 mg/L Mn, was shown to increase the growth and leaf enzymatic activities in *Myriophyllum verticillatum* under 400 mg/L Mn stress condition. The bacterium was also able to increase the Mn content in the stems and leaves of the inoculated plants by 36.4% and 54.7% , respectively, compared to non-inoculated plants (Tang et al. 2020). In a study to assess bacteria with potential to enhance growth and metal accumulation in hyperaccumulator *Alnus firma* (Babu et al. 2013), the bacterium identified as *Bacillus thuringiensis* GDB-1 had better capacity and increased significantly the biomass, chlorophyll content, and accumulation of As, Pb, Cu, Ni, and Zn in the *A. firma* seedlings. A summary of different species of *Bacillus* with plant growth promoting and heavy metal tolerant activity applied for growth enhancement and bioremediation of heavy metal in plant is presented in Tables 14.1 and 14.2.

In our recent study, one of the most promising and potential heavy metal tolerant plant growth-promoting isolate, identified as CCB-MBL5001 *Bacillus cereus* 2M1, was tested for its ability to alleviate metal stress and improve the growth of rice seedlings under Pb stress. Sterilized rice seeds (treated with or without 2M1 inoculation) were sown in sterile petri plates before uniformly germinated seeds were transferred aseptically to test tubes containing Yoshida medium supplemented with 0, 100 and 150 mg/L Pb. For both inoculated and non-inoculated seedlings, all growth parameters were noted to decline as affected by increasing concentrations of metal tested. However, better improvement in plant growth parameters were observed for seedlings inoculated with the bacteria compared to the control (non-inoculated, supplied with similar Pb concentrations) (Table 14.3). On the effects on photosynthetic pigments, the bacterial inoculated treatments significantly increased chlorophyll a, chlorophyll b, and total chlorophyll contents in the rice seedlings despite the occurrence of high metal stress (Fig. 14.1). Increase in the chlorophyll contents due to bacterial inoculation was not only found under Pb stress but also under non-stressed (control) conditions. Toxicity of heavy metal can affect photosynthesis by causing distortion in the ultrastructure of chloroplast, preventing the synthesis of photosynthetic pigment in chlorophyll content and enzymes of Calvin cycle. To assess the extent of oxidative stress and degree of damage caused by the heavy metal (Pb), the electrolyte leakage was estimated as presented in Fig. 14.2a. Stress resulting from the tested metal was shown to cause oxidative stress in the leaves of the host plants, which increased in response to increasing concentrations of the metal. However, there was significant decrease in the electrolyte leakage in 2M1 inoculated plants, which is a clear indication in the stability of the membrane. The % EL was decreased by 58% under the highest concentration of Pb, and the

Table 14.1 Plant growth enhancement and bioremediation of heavy metal in crop plants as influenced by plant growth promoting *Bacillus* species

Bacillus species	Plant species	Heavy metal	Function/activity	Condition	References
B. thuringiensis HC-2	Radish plant	Cd and Pb	Decreased Cd (28–94%) and Pb (22–63%) content in radish; increased dry weight of roots (18.4–22.8%) and leaves (37.8–39.9%)	Field	Li et al. (2019)
B. cereus MG257494.1	Sorghum	Cu, Cd, Zn and Pb	Decreased metal uptake; increased growth and biomass, antioxidant enzymes activity, and photosynthetic pigments	Pot	El-Meihy et al. (2019)
B. subtilis BM2	Wheat	Ni and Pb	Decreased metal uptake; improved growth parameters; relieved metal stress; decreased in proline, malondialdehyde content, and antioxidant enzymes activities	Pot	Rizvi et al. (2019)
P. mucilaginosus	Alfalfa	Cu	Increased nutrient content in plants; improved plant growth; increased antioxidant enzymes activities; improved growth and Cu uptake	Pot	Ju et al. (2019)
Bacillus sp. PSB10	Chickpea	Cr	Improved growth and biomass; increased nodulation and chlorophyll content; reduced Cr uptake by chickpea plants	Pot	Ahmad and Saghir (2010)

lower percentages of EL in the inoculated rice plants proved metal stress alleviation by the microbe to the plant. Plants are always being exposed to several stress factors in the field which can include heavy metal, that can affect their growth, development, and productivity. The adverse effects generally induce the accumulation of reactive oxygen species (ROS), which can cause severe oxidative damage including electrolyte leakage to plants. The balance between the production and detoxification of ROS is usually sustained by enzymatic and nonenzymatic antioxidants. The catalase (CAT) and superoxide dismutase (SOD) enzymes activities determined in the leaves of rice seedlings were higher in plants inoculated with 2M1 compared to their uninoculated counterparts (Fig. 14.2b and c). It was also observed that the inoculated controls without any heavy metal had the least activities. Increase in CAT and SOD activities by bacterial inoculation indicated strong response towards coping with oxidative stress generated by exposure to the heavy metal.

Table 14.2 Influence of *Bacillus* species on growth enhancement of hyperaccumulator plants and phytoremediation potentials of soils contaminated with heavy metal

Bacillus species	Plant species	Heavy metal	Function/activity	Condition	References
B. cereus HM5 *B. thuringiensis* HM7	*Broussonetia papyrifera*	Mn	Higher growth and biomass; increased accumulation of Mn by plant; reduced malondialdehyde and antioxidant activities in leaves	Pot	Huang et al. (2020)
B. thuringiensis GDB-1	*Alnus firma*	As, Ni, Cu, Pb and Zn	Increased growth and biomass, chlorophyll content, and heavy metal accumulation	Pot	Babu et al. (2013)
B. cereus WSE01	*Myriophyllum verticillatum*	Mn	Improved growth; increased leaf enzymatic activity; increased Mn accumulation in stems (36.4%) and leaves (54.7%)	Hydroponic culture	Tang et al. (2020)
B. subtilis SJ-101	*Brassica juncea*	Ni	Decreased Ni toxicity; enhanced Ni accumulation in B. juncea	Pot	Zaidi et al. (2006)
B. subtilis KP717559	*B. juncea*	Cd, Cr, and Ni	Increased growth and biomass; increased metal accumulation	Pot	Ndeddy Aka and Babalola (2016)
B. cereus T1B3	*Vetiveria zizanoides*	Cr and Fe	Enhanced growth and biomass; enhanced Cr and Fe accumulation	Pot	Nayak et al. (2018)

Table 14.3 Growth parameters of rice seedlings inoculated with locally isolated heavy metal tolerance *B. cereus* 2M1 under Pb stress condition

	Treatments		Plant growth parameter					
	Pb (mg/L)	Inoculation (2M1)	Root length (cm)	Shoot length (cm)	Root fresh weight (mg)	Shoot fresh weight (mg)	Root dry weight (mg)	Shoot dry weight (mg)
1	0	−	7.50 ± 0.29[a]	25.67 ± 2.90[a]	50.00 ± 0.00[a]	103.33 ± 3.33[a]	3.63 ± 0.58[ns]	19.10 ± 0.55[a]
2	0	+	7.83 ± 0.44[a]	26.50 ± 3.28[a]	50.00 ± 0.00[a]	106.67 ± 3.33[a]	3.80 ± 0.55[ns]	17.87 ± 1.49[a]
3	100	−	5.83 ± 0.44[bc]	15.73 ± 0.37[bc]	40.00 ± 5.77[ab]	73.33 ± 3.33[bc]	3.30 ± 0.44[ns]	8.33 ± 0.20[b]
4	100	+	5.33 ± 0.33[c]	17.03 ± 0.26[b]	43.33 ± 6.67[a]	80.00 ± 5.77[b]	3.50 ± 0.29[ns]	10.26 ± 0.68[b]
5	150	−	5.33 ± 0.60[c]	10.83 ± 0.60[c]	26.67 ± 3.33[c]	56.67 ± 6.67[c]	2.83 ± 0.44[ns]	7.97 ± 0.55[b]
6	150	+	7.07 ± 0.58[ab]	13.83 ± 0.44[bc]	40.00 ± 5.77[ab]	73.33 ± 3.33[bc]	3.57 ± 0.30[ns]	9.83 ± 0.95[b]

Values are presented as means ± standard error. Values with different superscripts along the columns are significantly different ($p < 0.05$)
Notes: − = uninoculated; + = inoculated; ns = not significant

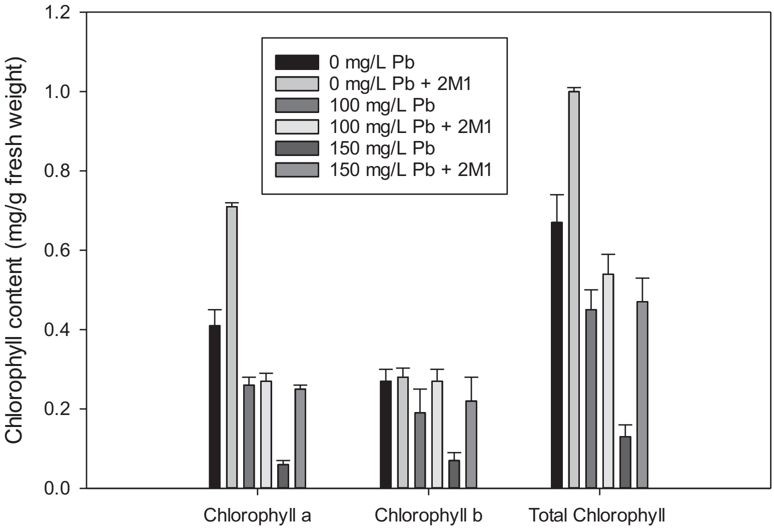

Fig. 14.1 Chlorophyll content in rice seedlings inoculated with locally isolated heavy metal tolerant *B. cereus* 2M1 after 15 days of growth under Pb stress and aseptic conditions

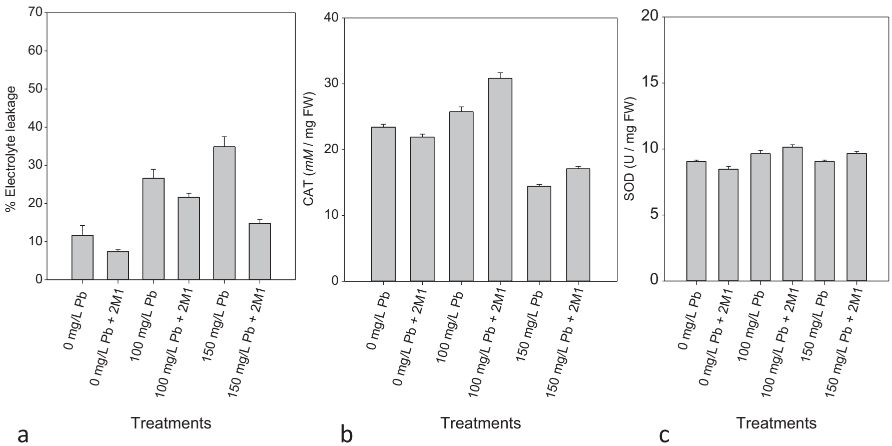

Fig. 14.2 Electrolyte leakage (**a**) CAT activity (**b**) and SOD activity (**c**) in rice seedlings inoculated with locally isolated heavy metal tolerant *B. cereus* 2M1 after 15 days of growth under Pb stress and aseptic conditions

14.7 Conclusions and Future Perspective

Bacilli have multiple beneficial traits, which mediate better growth and development of crop either directly or indirectly through their different plant growth-promoting activities and heavy metal tolerance enhancement. This has great application towards growth enhancement and bioremediation of heavy metal in crop plants. Information made available in this chapter are testament of great potential of

Bacillus spp. in growth enhancement and bioremediation of heavy metal in crop plants. However, most of the experiments were carried out under control conditions (lab or pot experiments), with paucity of information from field trials. Though the *Bacillus* group is one of the most commercially exploited bacteria in the agrobiotechnology industry, its potential has still not been realized sufficiently, and thus, the emphasis should be towards translating the relevant technologies from laboratory to the real world situation in the field (Saxena et al. 2019). More *Bacillus*-plant physiological studies are required to have better understanding of the *Bacillus* spp. mediated mitigation mechanisms against heavy metal stresses in plants.

References

Ahmad P, Saghir M (2010) Bacillus species enhance growth parameters of chickpea (Cicer arietinum L.) in chromium stressed soils. Food Chem Toxicol 48(11):3262–3267. https://doi.org/10.1016/j.fct.2010.08.035

Akinrinlola RJ, Yuen GY, Drijber RA, Adesemoye AO (2018) Evaluation of Bacillus strains for plant growth promotion and predictability of efficacy by in vitro physiological traits. Int J Microbiol

Amir HG, Shamsuddin ZH, Halimi MS, Marziah M, Ramlan MF (2005) Enhancement in nutrient accumulation and growth of oil palm seedlings caused by PGPR under field nursery conditions. Commun Soil Sci Plant Anal 36(15-16):2059–2066

Ashraf MA, Hussain I, Rasheed R, Iqbal M, Riaz M, Arif MS (2017) Advances in microbe-assisted reclamation of heavy metal contaminated soils over the last decade: A review. J Environ Manage 198:132–143. https://doi.org/10.1016/j.jenvman.2017.04.060

Babu AG, Kim JD, Oh BT (2013) Enhancement of heavy metal phytoremediation by Alnus firma with endophytic Bacillus thuringiensis GDB-1. J Hazard Mater 250–251:477–483. https://doi.org/10.1016/j.jhazmat.2013.02.014

Bachate SP, Nandre VS, Ghatpande NS, Kodam KM (2013) Chemosphere Simultaneous reduction of Cr (VI) and oxidation of As (III) by Bacillus firmus TE7 isolated from tannery effluent. Chemosphere 90(8):2273–2278. https://doi.org/10.1016/j.chemosphere.2012.10.081

Bellogín RA, Cerro P, Espuny MR, Pérez-monta F, Ollero FJ, Cubo T (2014) Plant growth promotion in cereal and leguminous agricultural important plants: From microorganism capacities to crop production. Microbiol Res 169:325–336. https://doi.org/10.1016/j.micres.2013.09.011

Bhattacharyya C, Bakshi U, Mallick I, Mukherji S, Bera B, Ghosh A (2017) Genome-guided insights into the plant growth promotion capabilities of the physiologically versatile Bacillus aryabhattai strain AB211. Front Microbiol 8(MAR):1–16. https://doi.org/10.3389/fmicb.2017.00411

Borriss, R. (2014). Use of plant-associated Bacillus strains as biofertilizers and biocontrol agents in agriculture (Issue June). https://doi.org/10.1007/978-3-642-20332-9

Ding Y, Wang J, Liu Y, Chen S (2005) Isolation and identification of nitrogen-fixing Bacilli from plant rhizospheres in Beijing region. J Appl Microbiol:1271–1281. https://doi.org/10.1111/j.1365-2672.2005.02738.x

Dixit R, Wasiullah Malaviya D, Pandiyan K, Singh UB, Sahu A, Shukla R, Singh BP, Rai JP, Sharma PK, Lade H, Paul D (2015) Bioremediation of heavy metal from soil and aquatic environment: An overview of principles and criteria of fundamental processes. Sustainability (Switzerland) 7(2):2189–2212. https://doi.org/10.3390/su7022189

El-Meihy RM, Abou-Aly HE, Youssef AM, Tewfike TA, El-Alkshar EA (2019) Efficiency of heavy metal-tolerant plant growth promoting bacteria for alleviating heavy metal toxicity on sorghum. Environ Exp Bot 162(February):295–301. https://doi.org/10.1016/j.envexpbot.2019.03.005

Fun CP, Rathinam X, Amir HG, Subramaniam S (2016) Characterization of a native Bacillus thuringiensis isolates from Malaysia that produces exosporium-enclosed parasporal inclusion. Emirate J Food & Agric 28(9):653–659
Gardener BBM (2004) Ecology of Bacillus and Paenibacillus spp. in Agricultural Systems. Phytopathology 94(December 2004):1252–1258. https://doi.org/10.1094/PHYTO.2004.94.11.1252
Halim MA, Rahman AY, Sim KS, Yam HC, Rahim AA, Amir HG, Najimudin N (2016) Genome sequence of a Gram-positive diazotroph, Paenibacillus durus type strain ATCC 35681. Genome Announc 4(1):e00005–e00016. https://doi.org/10.1128/genomeA.00005-16
Hashem A, Tabassum B, Fathi E, Allah A (2019) Bacillus subtilis : A plant-growth promoting rhizobacterium that also impacts biotic stress. Saudi J Biol Sci 26(6):1291–1297. https://doi.org/10.1016/j.sjbs.2019.05.004
Huang H, Zhao Y, Fan L, Jin Q, Yang G, Xu Z (2020) Improvement of manganese phytoremediation by Broussonetia papyrifera with two plant growth promoting (PGP) Bacillus species. Chemosphere 260:127614. https://doi.org/10.1016/j.chemosphere.2020.127614
Islam T, Rahman M, Pandey P, Boehme MH, Haesaert G (2019) Bacilli and agrobiotechnology: Phytostimulation and Biocontrol, vol 2. Springer Nature Switzerland AG
Ju W, Liu L, Fang L, Cui Y, Duan C, Wu H (2019) Impact of co-inoculation with plant-growth-promoting rhizobacteria and rhizobium on the biochemical responses of alfalfa-soil system in copper contaminated soil. Ecotoxicol Environ Saf 167(May 2018):218–226. https://doi.org/10.1016/j.ecoenv.2018.10.016
Kapahi M, Sachdeva S (2019) Bioremediation options for heavy metal pollution. J Health & Pollut 9(24). https://doi.org/10.5696/2156-9614-9.24.191203
Khalid S, Shahid M, Bibi I, Shah AH, Niazi NK (2018) A review of environmental contamination and health risk assessment of wastewater use for crop irrigation with a focus on low and high-income countries. Int J Environ Res Public Health 15(895):1–36. https://doi.org/10.3390/ijerph15050895
Kong Z, Glick BR (2017) The role of plant growth-promoting bacteria in metal phytoremediation. Adv Microb Physiol 71(July):97–132. https://doi.org/10.1016/bs.ampbs.2017.04.001
Li Z, Wang P, Yue X, Wang J, Ren B, Qu L (2019) Effects of Bacillus thuringiensis HC-2 combined with biochar on the growth and Cd and Pb accumulation of radish in a heavy metal-contaminated farmland under field conditions. Int J Environ Res Public Health 16
Li M, Gao X, Li C, Yang C, Fu C, Liu J, Wang R (2020) Isolation and identification of chromium reducing Bacillus Cereus species from chromium-contaminated soil for the biological detoxification of chromium. Int J Environ Res Public Health Vi
Ma Y, Rajkumar M, Zhang C, Freitas H (2016) Beneficial role of bacterial endophytes in heavy metal phytoremediation. J Environ Manage 174:14–25. https://doi.org/10.1016/j.jenvman.2016.02.047
Mia AB, Shamsuddin ZH, Wahab Z, Marziah M (2010) Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. Aust J Crop Sci 4(2):85–90
Minaxi NL, Yadav RC, Saxena J (2012) Characterization of multifaceted Bacillus sp. RM-2 for its use as plant growth promoting bioinoculant for crops grown in semi arid deserts. Appl Soil Ecol 59:124–135. https://doi.org/10.1016/j.apsoil.2011.08.001
Mishra J, Singh R, Arora NK (2017) Alleviation of heavy metal stress in plants and remediation of soil by rhizosphere microorganisms. Front Microbiol 8(SEP):1–7. https://doi.org/10.3389/fmicb.2017.01706
Nascimento FX, Hernández AG, Glick BR, Rossi MJ (2020) Plant growth-promoting activities and genomic analysis of the stress-resistant Bacillus megaterium STB1, a bacterium of agricultural and biotechnological interest. Biotechnol Report 25:1–9. https://doi.org/10.1016/j.btre.2019.e00406
Nayak AK, Panda SS, Basu A, Dhal NK (2018) Enhancement of toxic Cr (VI), Fe, and other heavy metal phytoremediation by the synergistic combination of native Bacillus cereus strain and Vetiveria zizanioides L. Int J Phytoremediation 20(7):682–691. https://doi.org/10.1080/15226514.2017.1413332

Ndeddy Aka RJ, Babalola OO (2016) Effect of bacterial inoculation of strains of Pseudomonas aeruginosa, Alcaligenes feacalis and Bacillus subtilis on germination, growth and heavy metal (Cd, Cr, and Ni) uptake of Brassica juncea. Int J Phytoremediation 18(2):200–209. https://doi.org/10.1080/15226514.2015.1073671

Ojuederie OB, Babalola OO (2017) Microbial and plant-assisted bioremediation of heavy metal polluted environments: a review. Int J Environ Res Public Health 14(12). https://doi.org/10.3390/ijerph14121504

Oves M, Saghir KM, Huda QA, Nadeen FM, Almeelbi T (2016) Heavy metal: Biological importance and detoxification strategies. J Bioremed Biodegr 7(2). https://doi.org/10.4172/2155-6199.1000334

Radhakrishnan R, Hashem A, Abd Allah EF (2017) Bacillus: A biological tool for crop improvement through bio-molecular changes in adverse environments. Front Physiol 8(SEP):1–14. https://doi.org/10.3389/fphys.2017.00667

Rizvi A, Ahmed B, Zaidi A, Khan MS (2019) Heavy metal mediated phytotoxic impact on winter wheat: oxidative stress and microbial management of toxicity by Bacillus subtilis BM2. RSC Adv:6125–6142. https://doi.org/10.1039/c9ra00333a

Salwani S, Amir HG, Najimudin N (2012) Evidence of diazotrophic symbionts in the leguminous cover crop mucuna bracteata. Pertanika J Tropic Agric Sci 35(3):537–552

Saxena AK, Kumar M, Chakdar H, Anuroopa N, Bagyaraj DJ (2019) Bacillus species in soil as a natural resource for plant health and nutrition. J Appl Microbiol:1583–1594. https://doi.org/10.1111/jam.14506

Syed S, Chinthala P (2015) Heavy metal detoxification by different Bacillus species isolated from solar salterns. Scientifica 2015:1–8. https://doi.org/10.1155/2015/319760

Tang Y, Kang H, Qin Z, Zhang K, Zhong Y, Li H, Mo L (2020) Significance of manganese resistant Bacillus cereus strain WSE01 as a bioinoculant for promotion of plant growth and manganese accumulation in Myriophyllum verticillatum. Sci Total Environ 707:135867. https://doi.org/10.1016/j.scitotenv.2019.135867

Titah HS, Abdullah SRS, Idris M, Anuar N, Basri H, Mukhlisin M, Tangahu BV, Purwanti IF, Kurniawan SB (2018) Arsenic Resistance and Biosorption by Isolated Rhizobacteria from the Roots of Ludwigia octovalvis. Int J Microbiol. https://doi.org/10.1155/2018/3101498

Tiwari S, Lata C (2018) Heavy metal stress, signaling, and tolerance due to plant-associated microbes: an overview. Front Plant Sci 9(April):1–12. https://doi.org/10.3389/fpls.2018.00452

Weissmannová HD, Mihoˇcová S, Chovanec P, Pavlovský J (2019) Potential ecological risk and human health risk assessment of heavy metal pollution in industrial affected soils by coal mining and metallurgy in Ostrava, Czech Republic. Int J Environ Res Public Health 16(2019)

Wu J, Kamal N, Hao H, Qian C, Liu Z, Shao Y, Zhong X, Xu B (2019) Endophytic Bacillus megaterium BM18-2 mutated for cadmium accumulation and improving plant growth in Hybrid Pennisetum. Biotechnol Report 24. https://doi.org/10.1016/j.btre.2019.e00374

Yahaghi Z, Shirvani M, Nourbakhsh F, de la Peña TC, Pueyo JJ, Talebi M (2018) Isolation and characterization of Pb-solubilizing bacteria and their effects on pb uptake by Brassica juncea: Implications for microbe-assisted phytoremediation. J Microbiol Biotechnol 28(7):1156–1167. https://doi.org/10.4014/jmb.1712.12038

Zaidi S, Usmani S, Singh BR, Musarrat J (2006) Significance of Bacillus subtilis strain SJ-101 as a bioinoculant for concurrent plant growth promotion and nickel accumulation in Brassica juncea. Chemosphere 64:991–997. https://doi.org/10.1016/j.chemosphere.2005.12.057