



# Rhizobacteria for Reducing Heavy Metal Stress in Plant and Soil

# 10

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## Abstract

The intensity of pollution expansion is increasing day by day of which heavy metal pollution has taken the center stage of discussion since the last few decades. Heavy metals have direct detrimental effect on our ecosystem in general and on the agroecosystem in particular, thereby proving to be hazardous for plants, animals, and microbes. One of the most common, low-cost, and eco-friendly strategies that can be employed to counter this problem effectively is through bioremediation. However among several types of bioremediation, microbial bioremediation with the use of rhizobacteria is best suited for alleviating heavy metal stresses in the agroecosystem.

## Keywords

Heavy metals · Rhizobacteria · PGPR · Bioremediation

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179

## 10.1 Introduction

There exists a lot of misperception over the classification and definition of heavy metals. Still scientific groups have not reached into any consensus regarding this issue. In a report published in the International Union of Pure and Applied Chemistry (IUPAC), Duffus (2002) raised questions over the usage of the term “heavy metals” and its classifications. Therefore he suggested undertaking a much broader approach while classifying heavy metals based on the periodic table. In agreement with his views, Appenroth (2010) proposed for considering three groups of elements (transition elements, rare earth metals, and borderline elements) as heavy metals from the periodic table after thoroughly studying their chemical properties.

Keeping all these discussions aside, however, the most commonly followed definition of heavy metals is “These are the elements with an atomic weight between 63.5 and 200.6 followed by a specific gravity of more than 5.0” (Srivastava and Majumder 2008). In simple terms, we can say that they are heavier than water by five times or are having an atomic density  $>4 \text{ g/cm}^3$  (Durube et al. 2007; Mahamood et al. 2012). They can also be defined as the block of all metals in Groups 3–16 that are present in period 4 and above, i.e., periods 5, 6, and 7 (Hawkes 1997). The term heavy metals in a broader sense are often used whenever there arises some implication for toxicity. As heavy metals are present in very minute quantity, i.e.,  $1 \mu\text{g kg}^{-1}$ , these are often represented as trace elements (Tchounwou et al. 2012). Some of these trace metals are beneficial for plants (Zn, Mn, Fe, Cu, B, and Mo), while others are non-beneficial (Se and Co), and the rest (As, Hg, Pb, Cr, Cd, and Ni) are toxic (He et al. 2005).

### 10.1.1 Current Status of Heavy Metal Pollution

Pollution of heavy metals has been seen everywhere across the earth (lithosphere, atmosphere, and hydrosphere). It has been escalated to such an extent that it can be found even on the most extreme climatic conditions on earth starting from Mount Everest (Yeo and Langley-Turnbaugh 2010) to the deep ocean floor (Humbatov et al. 2015) and also underneath the topsoil layer (Wuana and Okieimen 2011; Su et al. 2014). Bioaccumulation of these metals can be seen on food items like milk (Tunegova et al. 2016), vegetables (Agrawal et al. 2007; Mishra and Tripathi 2008), fishes (Ebrahimpour et al. 2011; Abarshi et al. 2017), and livestock (Rajaganapathy et al. 2011; Okareh and Oladipo 2015). Rampant pollution had led to their worldwide distribution across every continent. Be it Asia (Rajindiran et al. 2015; Chen et al. 2015; Ghorbani et al. 2015) or Africa (Yabe et al. 2010), their presence can be felt everywhere. Rapid industrialization has also escalated their concentration in developed portions of the world like Europe (Panagos et al. 2013; Toth et al. 2016), Australia (Hart and Lake 1987), and South America (Smolders et al. 2003; Eichler et al. 2015). However, their presence in Antarctica seems to be quite surprising as it is so far uninhabited and unexplored as compared to the rest of the world (Evans et al. 2000; Santos et al. 2005). These

things reflect the true situation of heavy metal pollution, thus a much needed eye-opener for us to save our ecosystem from further destruction.

Heavy metals are also found above permissible limits in our day-to-day utility commodities like food items (Mahaffey et al. 1975), soft drinks (Bingol et al. 2010; Godwill et al. 2015), and cosmetics (Borowska and Brzoska 2015). In some of the worst affected countries like India and Bangladesh, arsenic (As) is present above permissible limits in rice grains (Sinha and Bhattacharyya 2014; Meharg and Rahman 2003). Rice being the staple food in these countries leads to direct intake of arsenic. Not only in rice but also arsenic in cereals, pulses, vegetables, and forage crops has been reported by several researchers (Sharma et al. 2007; Santra et al. 2013). A regular dietary intake of these arsenic-contaminated food items (Signes et al. 2008) is a direct threat to one's life. Therefore, different regulatory agencies like the World Health Organization (WHO), European Food Safety Authority (EFSA), and Agency for Toxic Substances and Disease Registry (ATSDR) have prescribed the maximum intake capacity of heavy metals as mentioned in Table 10.1.

Arsenic among all these heavy metals is ranked among the top ten hazardous chemicals by WHO. Besides this, it is also ranked number 1 by ATSDR (2017) on its substance priority list followed by lead and mercury. Lead till now is probably the most well-studied occupational toxin causing about 0.6% of all diseases worldwide (Gidlow 2004). More than 120 million people worldwide come under the threat lead toxicity with developing nations being the most affected (Venkatesh 2009).

The direct impact of heavy metal contamination is seen in soil and groundwater. The European Commission's report on soil contamination and their impact on human health stated that heavy metals are the most frequently occurring contaminants on soil (35%) and groundwater (31%). Soils (around 33%) all over the world are facing serious heavy metal contamination problem (Roslan et al. 2016). Say for China, around 19.40% of Chinese farmland is facing heavy metal

**Table 10.1** Permissible limits of different heavy metals set by EFSA (European Food Safety Authority), WHO (World Health Organization), and ATSDR (Agency for Toxic Substances and Disease Registry)

Metals	EFSA (2006)	WHO-FAO (1995)	ATSDR (2018)
Ni	2.8 µg/kg of body weight (TDI)	<100 µg/day	0.0002 mg/m <sup>3</sup>
Hg	1.3 µg/kg of body weight (TWI)	5 µg/kg of body weight per week	0.0002 mg/m <sup>3</sup>
Cr	0.3 mg/kg of body weight (TDI)	33 µg/day	0.005 mg/kg/day
Cd	2.5 µg/kg of body weight (TWI)	7 µg of cadmium/kg of body weight per week	0.0005 mg/kg/day
As	<15 µg/kg of body weight (TWI)	<200 µg/day	0.005 mg/kg/day
Pb	<25 µg/kg of body weight (TWI)	25 µg/kg of body weight per week for adults	

*TDI* tolerable daily intake, *TWI* tolerable weekly intake

pollution (Zhang et al. 2015). Due to soil pollution, a loss of more than 10 billion US dollars is being incurred from over 10 million polluted sites out of which 50% contaminants happen to be heavy metals (He et al. 2015). Agricultural pesticides are one of the main sources of arsenic contamination in soil. A total of around 80–90% arsenic produced annually finds its way into soil through these chemicals (Nriagu and Pacyna 1988). Hutton and Symon (1986) reported that annually 1637 tons of lead and 111 tons of arsenic are being deposited into the arable soils of the United Kingdom through anthropogenic sources.

Atmospheric pollution of heavy metals after soil is the next biggest concern for researchers. About 30% of mercury per annum is released from anthropogenic sources into the atmosphere of which 50% comes from Asia alone (UNEP 2013). Excessive release of mercury into the air transports them to North America by wind, accounting for 5–36% of mercury deposition in the United States (Jaffe et al. 2005). Due to its long-range transport ability, even the Arctic region is also polluted from mercury contamination (Ilyin et al. 2004). Other than mercury, cadmium also contributes significantly to atmospheric heavy metal pollution. It has been reported that Spain and France equally contribute (i.e., 16%) for cadmium emission in Europe's air (Dinis and Fiuza 2011). In recent times, Indian cities also show the presence of heavy metals in their atmosphere, often exceeding the maximum permissible limits (Chaudhari et al. 2012; Dey et al. 2014).

Groundwater heavy metal contamination is also an equally important global concern like soil and air heavy metal pollution. Among all heavy metals, arsenic contamination in groundwater is most noticed with South Asian countries like Bangladesh and India being worst affected (Ravenscroft et al. 2005; Pal et al. 2009). All over the world, nearly 130 million people come under the threat of arsenic contamination by drinking As-contaminated water, which is often above the prescribed limit (10 ppb) set by WHO (UNICEF 2008). Majority of these populations are inhabitants of two countries, i.e., Bangladesh (35–77 million) and India (12 million from the state of West Bengal alone), making them globally the worst hit countries (Smith et al. 2000; Ravenscroft et al. 2009). In India there are seven major states (West Bengal, Assam, Uttar Pradesh, Chhattisgarh, Bihar, Jharkhand, and Manipur) which find arsenic contamination in their groundwater (Chakraborti et al. 2017). The regulatory limits of heavy metals in drinking water prescribed by different agencies have been stated in Table 10.2.

### 10.1.2 Sources of Heavy Metal Pollution

Heavy metal contamination in the environment occurs through natural and anthropogenic means (Chen et al. 2009; INSA 2011). Heavy metals are nondegradable, due to which they are persistent in our environment and in the course of time get released into the soil, water, and air (Zaharescu et al. 2009; Aksu 2015; Van et al. 2016; Drira et al. 2017). During weathering and soil formation processes, they are released from rocks (metamorphic, sedimentary, and magmatic rocks) and minerals (oxides, hydroxides, and clay minerals) into the environment (Brad 2005). The fate

**Table 10.2** Minimum prescribed limits for heavy metals in drinking water set by different regulatory agencies

Heavy metals	BIS (2012) in mgL <sup>-1</sup>	WHO (2017) in mgL <sup>-1</sup>	EPA (2001) in mgL <sup>-1</sup>
Selenium	0.01	0.04	0.01
Cadmium	0.003	0.003	0.005
Lead	0.01	0.01	0.05
Mercury	0.001	0.006	0.001
Nickel	0.02	0.07	
Arsenic	0.01	0.01	0.05
Chromium	0.05	0.05	0.05
Antimony	–	0.02	5

of these metals on soil is governed by the type of parent materials and physiochemical properties of soil (Abdelilah et al. 2010; Roozbahani et al. 2015). Natural phenomena like volcanic eruptions, forest fires, and soil erosions play a major role in their distribution (Bielicka et al. 2005; Akpor et al. 2014). In aquatic systems, sediments are the chief storehouse of heavy metals, governing their overall distribution and transformation processes in water bodies (Wu et al. 2014). The level of heavy metals is often well regulated and rarely crosses their limits in natural environment.

However in contrast to natural sources, anthropogenic sources are more responsible for elevation of heavy metal concentration in natural environment (Xu et al. 2014). Human-driven activities like mining, intensive agricultural practices, and road constructions driven by urbanization and industrialization act as the perfect catalyst for their release into natural environment (Imperato et al. 2003; Liao et al. 2018). Intensive agricultural practices like excessive usage of pesticides and fertilizers coupled with sewage water for irrigation have led to the accumulation of heavy metals in cultivated soils (Sidhu 2016). Furthermore, runoff water passing through highways during rainfall contains heavy metals (Turer et al. 2001). Besides these, polyvinyl chloride (PVC) products, chargeable batteries, brake linings, tires, color pigments, furnace dusts, etc. are some other potential sources of heavy metals (Oves et al. 2016). Comprehensive descriptions for anthropogenic sources of heavy metals are listed in Table 10.3.

## 10.2 Effects of Heavy Metals on Life Forms

Due to their persistent nature, heavy metals accumulate in our body resulting in several health issues (Sharma et al. 2007; Garg et al. 2014). Heavy metals enter our body through food, air, and water. However the chief entry route of heavy metals into our body is through food (Darwish et al. 2015; Yadav et al. 2017). Regular intake of heavy metal-contaminated food can retard growth and weaken our immune system (Singh and Kalamdhad 2011). Alongside food, they can also make entry through the skin and air (Liang et al. 2017). Entry of these metals through food chain causes their bioaccumulation and paves the path for several cardiovascular,

**Table 10.3** Different heavy metals and their anthropogenic sources

Heavy metals	Anthropogenic sources	References
Arsenic (As)	Herbicides, pesticides, inorganic fertilizers, coal and petroleum combustion, nonferrous metal smelting, mining, poultry litter, sewage sludge, fly ash, wood preservatives, desiccants, feed additives, pharmaceutical industries, glass industry, pigments, cigarettes, semiconductor manufacturing, cotton ginning	Bellows (2005), Hamzah et al. (2013), Arunakumara et al. (2013), Chung et al. (2014), ATSDR (2007a, b), and Rice et al. (2002)
Cadmium (Cd)	Cigarettes, fertilizers, polymer industry, varnished industry, coatings, pigments and coloring agents, stabilizers, electronic waste (e-waste), batteries, phosphate fertilizers, smelting and refining of nonferrous metals, fossil fuel combustion, liming agents, manures, sewage sludge	Hutton (1983), Sugita et al. (2001), Piade et al. (2015), and Rosemary et al. (2014)
Chromium (Cr)	Paints and pigments, leather industry, stainless steel and iron production, textile industry, porcelain and ceramics manufacturing, chrome alloy production and electroplating, wood preservatives, coal and oil combustion, chemical industry	Saha et al. (2011), ATSDR (2012a, b), and Chung et al. (2014)
Lead (Pb)	Battery, pigments, plastics, rubber industry, smelting plants, ceramics, petrol, gasoline, solid waste combustion, cigarettes	Zeitoun and Mehana (2014), Ashraf (2011), and Mielke et al. (2001)
Mercury (Hg)	Coal burning, chlor-alkali plants, cement production, nonferrous smelting, waste incineration, refining, gold mining, chemical industry, pharmaceutical industries, fungicides, fluorescent and ultraviolet lamps	Rodrigues et al. (2006) and Naja and Volesky (2009)
Nickel (Ni)	Mining and smelting, ferrous and nonferrous metals production, battery, chemical industry, electroplating, petroleum processing, cement manufacturing, sewage sludge incineration, coal and oil combustion, nickel matte refining, steel production, nickel alloy production, vehicle emissions, fertilizer and organic manures, cement production, disinfectants manufacture	ATSDR (2005a, b)
Selenium (Se)	Coal and oil combustion, glass industry, semiconductor manufacturing, paint industry, mining and smelting, ceramics, refining, sewage sludge, photo cells, vulcanization of rubber, pharmaceutical industries, insecticides, herbicides, lubricants, xerography (photocopiers), animal feed additives, manufacture of inorganic pigments, phosphate fertilizers	ATSDR (2003)

nervous, kidney, and bone diseases (Rani and Goel 2009; Ji-yun et al. 2016). Health issues occurring due to heavy metals are enlisted in Table 10.4.

The term heavy metal is often used in context of toxicity, but it should be noted that not all heavy metals (like Mn, Cu, Zn, Fe, etc.) are harmful (Flora et al. 2008).

**Table 10.4** Impact of various heavy metals on human health

Heavy metals	Impact on human health	References
Arsenic	Arsenicosis (chronic arsenic toxicity), arteriosclerosis, laryngitis, respiratory diseases, nausea, vomiting, proteinuria, diarrhea, abdominal pain, anorexia, weight loss, pigmentation, neuritis, skin lesions, keratosis, melanosis, dermatosis, hypertension, bronchitis, oliguria, renal failure, affects heme biosynthesis, Anemia, leucopenia, low IQ in children, cancer (lungs, skin, kidney, bladder, liver, colon and nasal cancer), gastroenteritis, diabetes, neurobehavioral changes and abnormalities, peripheral neuropathy, increases fetal mortality rate, polyneuropathies, hallucinations, increases stillbirth, weakness and fatigue, edema, Bowen's disease	ATSDR (2007a, b), Singh et al. (2007), Hughes et al. (2011), Mazumder (2008), Pierce et al. (2010), Tchounwou et al. (2003), Silva et al. (2005), Florea and Busselberg (2006), and Rossman (2003)
Nickel	Severe lung damage, giddiness, headache, diarrhea, hematuria, allergic dermatitis, emphysema, nausea, pulmonary fibrosis, vomiting, vertigo, kidney problems, mucosal irritation, tachycardia, abdominal pain, muscular pain, asthma, bronchitis, dyspnea, cyanosis, cancer (lungs, nasal cavity, kidney, prostate, bone and laryngeal cancer)	ATSDR (2005a, b), Al-Fartusie and Mohssan (2017), and Das et al. (2008)
Cadmium	Hypertension, osteoporosis and osteomalacia, emphysema, testicular atrophy, muscular weakness, bronchiolitis, renal failure, olfactory dysfunction, increases fetal mortality, abdominal cramps, anosmia, memory loss, lymphocytosis, eosinophilia, nausea, vomiting, itai-itai disease, glucosuria, proteinuria, myocardial infarction, chronic rhinitis, cancer (kidney, lung, pancreas, urinary bladder, endometrium, breast, and prostate cancer)	ATSDR (2012a, b), Ayangbenro and Babalola (2017), Sharma et al. (2014), Notarachille et al. (2014), Singh and Kalamdhad (2011), and Wu et al. (2016a, b)
Lead	Headaches, hypertension, vomiting, nausea, depression, anxiety, reduced fertility and miscarriages, renal failure, hallucinations, Anemia, abdominal pain, gastrointestinal problems, high blood pressure, encephalopathy, hemoglobinuria, loss of appetite, loss of memory, intellectual disorders, behavioral problems, diarrhea, low IQ in children, constipation, lethargy, impairment of neurological development (ataxia), growth and mental retardation, cancer (lung, brain, kidney, and stomach cancer)	ATSDR (2007a, b), Sharp and Brabander (2017), Mamtani et al. (2011), Jan et al. (2015), Lee et al. (2018), Rousseau et al. (2007), Qu et al. (2018), and Patocka and Kuca (2016)

(continued)

**Table 10.4** (continued)

Heavy metals	Impact on human health	References
Mercury	Prenatal toxicity and damage, impaired sexual functions, proteinuria, edema, dermatitis, pneumonitis, gingivitis, insomnia, respiratory failure, deafness, mental retardation, blindness, dysarthria, cough, dyspnea, mercurial erythrim, insomnia, weight loss, renal tubular dysfunction and kidney failure, neuropsychiatry disorders, infertility, miscarriage, neuropsychiatry disorders, memory loss	ATSDR (1999), Clarkson (1992), Maqbool et al. (2017), Golding et al. (2013), and Eqani et al. (2016)
Chromium	Irritation to the nasal cavity, asthma and cough, dermatitis, epistaxis, pneumoconiosis, gastrointestinal problems, kidney and liver problems, hypochromic anemia, decrease in sperm count, hyperplasia, postnatal hemorrhage, abdominal pain, bloody diarrhea, cancer (lung and nasal cavity), renal failure, skin ulcers	ATSDR (2012a, b), Jomova and Valko (2011), and Ding and Shi (2002)
Selenium	Nausea, vomiting, tachycardia, diarrhea, selenosis (high level of se in blood), fatigue, hair loss, irritability, dermal and neurological effects	ATSDR (2003) and Fraga (2005)
Thallium	Hair loss (alopecia), vomiting, diarrhea, constipation, palmar erythema, anorexia, blindness, affects menstrual cycle, high blood pressure, joint pain, tachycardia, polyneuropathy, muscle weakness, disturbance in vision, paraesthesia, psychosis, depression, behavioral abnormalities, gastroenteritis, may cause death also. Affects respiratory, gastrointestinal, cardiovascular and male reproductive system	ATSDR (1992), Achparaki and Thessalonikeos (2012), Peter and Viraraghavan (2005), Cvjetko et al. (2010), Xiao et al. (2012), and Li et al. (2015)
Copper	Nausea, vomiting, diarrhea, severe headache, abdominal pain, hair loss, anemia, male infertility, coughing, sneezing, insomnia, convulsion, arthritis, attention deficit disorder, pulmonary fibrosis, jaundice, autism, prostatitis, renal failure, gastrointestinal problems, hypotension, bronze diabetes, liver damage	ATSDR (2004) and Ashish et al. (2013)
Zinc	Nausea, vomiting, respiratory disorder, diarrhea, coughing, abdominal pain, anemia, leukopenia, dyspnea, renal failure, gastroenteritis, conjunctivitis, skin damage (blisters and ulcers), hypertension, acute pneumonitis, pulmonary fibrosis, constipation, headache, insomnia, pharyngitis	ATSDR (2005a, b) and Plum et al. (2010)

Some of these heavy metals are part of several metabolic pathways, while the rest are toxic to our body (Mahurpawar 2015; Al-Fartusie and Mohssan 2017). Chromium, for instance, has dual functions in our body. In its low concentration, it is used in a number of metabolic processes (like fat and protein metabolism), while excess exposure causes several respiratory diseases (Sathawara et al. 2004).



In a similar way, copper is used for iron absorption and signaling. However when present in excess amount, it causes liver and kidney dysfunctions (Ashish et al. 2013). More often than not, we consider Zn (Zinc) as an essential element as it is part of numerous proteins and metalloenzymes. It is observed that excessive amount of Zn in our body may result in nausea and vomiting while its deficiency leads to neural disorders (Plum et al. 2010). Heavy metals are mutagenic and carcinogenic in nature (Silva et al. 2005; Fernandez-Luqueno et al. 2013). Heavy metal contamination causes a wide range of health issues related to developmental, gastrointestinal, dermal, respiratory, cardiovascular, immunological, and reproductive systems (Liu et al. 2013).

Heavy metals lead to oxidative stress, and to neutralize this effect, the cell produces antioxidants (catalase and superoxide dismutase) in its response. This balance is always maintained in our body, and any imbalances lead to altered gene expression, activation of signaling pathways, and production of cytokines (Salnikow et al. 2000; Leonard et al. 2004). Activation of metal-induced signaling pathways affects several signaling components (G-proteins, MAP kinases, tyrosine kinases, growth factor receptors, and nuclear transcription factors), thereby disrupting the normal functioning of the cell (Harris and Shi 2003; Flora et al. 2008). Researchers have also reported that heavy metal stresses induce apoptosis in cells (Wang and Shi 2001). Heavy metals cause cancer and are thus labeled as carcinogens (Galaris and Evangelou 2002). These metals damage DNA and cause mutation which leads to cancer (Durham and Snow 2006; Jadoon and Malik 2017), with lung and skin cancers being the most common among them (Harris and Shi 2003). However they also cause several other cancers like liver, kidney, bladder, prostate, lymphoma, leukemia, and breast (Pourahmad et al. 2003). The central nervous system (CNS) and hematopoietic system are also affected by the presence of these metals (Florea and Busselberg 2006). It has been reported that these metals are related to a wide range of neurological diseases like Wilson's disease (Cu), Parkinson's disease (Fe, Mn, and Cu), Alzheimer's disease (Cd), Hallervorden-Spatz disease (Fe), multiple sclerosis, polycythemia, Minamata disease (Hg), muscular dystrophy, sideroblastic anemia, itai-itai disease (Cd), and blackfoot disease (As), among others (Montgomery 1995; Khan et al. 2013; Jaishankar et al. 2014; Draszawka-Bolzan 2014; Min and Min 2016). Metal toxins alter the functioning of neurotransmitters like catecholamines and bring about behavioral changes in humans (Shukla and Singhal 1984; Inoue 2013). Premature aging can occur due to heavy metal toxicity, thus paving the path for occurrence of numerous diseases (Mudgal et al. 2010).

Like humans, plants too uptake heavy metals, and their entry points are root and leaves. They get deposited in the cell wall, plasma membrane, or cytoplasm after traveling through xylem by means of apoplastic and symplastic pathways (Shahid et al. 2015; Clemens and Ma 2016). Uptake of heavy metal by plants is greatly influenced by the type of plant species and the defense mechanisms followed by them to overcome its toxicity (Alves et al. 2016). In agriculture, there are a lot of crop plants which show phytotoxicity to these metals (Forster 1954; Benzarti et al. 2008). The attributes that are hampered by heavy metal toxicity are seed germination, yield, nutrient uptake, and nitrogen fixation (Athar and Ahmad 2002; Guala et al. 2010;

Sethy and Ghosh 2013). It has been observed that sometimes heavy metals besides competing with each other also try to compete with several other essential elements for their uptake, both at the cellular level and in the soil system (Krupa et al. 2002; Israr et al. 2011). For example, a certain concentration of As (arsenic) helps in the uptake of Mn, Cu, Fe, and P; however with its further increase in concentration, uptake of these metals decreases (Farnese et al. 2014).

There is significant reduction in the photosynthetic rate of plants due to heavy metal toxicity. This is due to the fact that these metals affect the enzymes of photosystem I and II which causes lower biomass production (Oves et al. 2016). Physiological and biochemical activities of plants like respiration, translocation, transcription, translation, mineral metabolisms, cell signaling, and cell cycle along with some developmental processes like flowering and embryogenesis are also affected (Ovecka and Takac 2014). Due to the presence of abiotic stresses (i.e., from heavy metals), lower root and shoot growth is observed in several crop plants which can be correlated with decrease in chlorophyll and protein content in these plants (Manios et al. 2002; John et al. 2009). Furthermore, heavy metal toxicity is dependent on plant growth stages (Cheng 2003; Peralta-Video et al. 2004).

Like humans, plants also produce reactive oxygen species (ROS) like  $H_2O_2$ ,  $OH^-$ ,  $^1O_2$ , and  $O_2^-$  and reactive nitrogen species (RNS) like nitric oxide and peroxynitrite  $ONOO^-$  and free radicals in response to oxidative stress caused by heavy metals (Zengin and Munzuroglu 2005; Moller et al. 2007). Oxidative stress results in cellular toxicity and leads to oxidative degradation of biomolecules like carbohydrates, proteins, lipids, and nucleic acids (Aras et al. 2012). Arsenic toxicity displays a variety of symptoms in plant like leaf defoliation, chlorosis, necrosis, reduced fertility, stunted growth, and senescence and under severe condition may also cause death (Gulz et al. 2005; Abbas et al. 2018). Phosphate metabolism in plants gets affected by arsenic as arsenate mimics phosphate ion and can get substituted in its place (Kaur et al. 2011). Besides this, magnesium ion in chlorophyll molecule may also be substituted by other heavy metals (Zurek et al. 2014). Likewise, cadmium also interferes with several plant processes like photosynthesis, transpiration, mineral nutrition (N, K, Ca, Mg, P, and Fe), stomatal opening, and antioxidant metabolism (Benavides et al. 2005; Nazar et al. 2012). Nickel plays an essential role in nitrogen metabolism and seed germination. However, Ni toxicity results in chlorosis and yellowing of leaves which finally affect the normal functioning of plant (Selvaraj 2018). In some cases, it is interesting to see that two heavy metals have additive effects on their toxicity in plants. For instance, in barley plant, it has been observed that the combined effect of copper and cadmium resulted in lower root and shoot growth (Zaltauskaite and Sliumpaite 2013).

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### 10.3 Heavy Metals and Microorganisms

Microorganisms also are subjected to heavy metal stress like any other life forms. Microbes (diatoms and microalgae) are often used for heavy metal pollution assessment and act as bioindicators (Sbihi et al. 2012; Djukic and Mandic 2018). Microbes

are very sensitive to heavy metals and exhibit this sensitivity even at species and strain level (Giller et al. 1998). Microbes from different habitats and groups exhibit varied level of heavy metal tolerance (Sadler and Trudinger 1967). Generally, fungi are said to be more tolerant than bacteria to these metals (Rajapaksha et al. 2004).

Soil when exposed with heavy metals for a prolonged period of time resulted in decreased microbial biomass and reduced microbial diversity and activity with further change in their genetic composition (Chen et al. 2014; Kuzniar et al. 2018). These metals also considerably influence the bacterial community structure as revealed from metagenomic studies (Yao et al. 2017). These metals enhance microbial growth in its lower concentration while when present in excess quantity are harmful for the cell by affecting its membrane integrity, destroying its cellular organelles, and damaging its genetic materials (Sengor et al. 2009). Furthermore, an increase in lag time brings about reduction in growth of microbial cells (Gikas et al. 2009). Physiological activities like respiration and metabolism are affected due to heavy metals resulting in lower production of soil enzymes (Xie et al. 2016). Further, reproduction of several fungal species also gets influenced by the presence of these metals. Baldrian (2003) reported that the reproductive stages of saprophytic and mycorrhizal fungus were more affected as compared to their vegetative stages.

Microbes growing in the presence of heavy metals show certain morphological changes like transformation from one form to another. Certain bacteria change their shape from rod to spherical in copper's presence (Sadler and Trudinger 1967). Similar findings have been reported in fungi where heavy metal induces certain morphological changes in fungal hyphae (Ali 2007). Soil-inhabiting fungus is also affected from these metals. Fungi play an important role in biodegradation process and biogeochemical cycles while influenced by the presence of heavy metals (Hartikainen et al. 2012; Khan et al. 2013). Nitrification, which is a crucial step in nitrogen cycle, is significantly inhibited by the presence of these metals (Park and Ely 2008; Hamsa et al. 2017). Microorganisms have the ability of uptaking heavy metals through certain metabolic or physiochemical pathways known as microbial biosorption. This metal uptake rate depends upon a wide array of factors like physiological state of cell, nature of growth medium, and type of microbes growing (Vijayadeep and Sastry 2014). By effectively utilizing this property, microbes can serve as a tool for alleviating heavy metal stress from the environment (Yamaji et al. 2016).

### 10.3.1 Bioremediation of Heavy Metal by Rhizobacteria

Soil pollutants can be extracted from the soil by employing several bioremediation techniques. Plant growth-promoting rhizobacteria (PGPR) are one of the better prospects for bioremediation of heavy metals in the rhizosphere. Rhizobacteria in combination with plants are more fruitful and provide better efficiency for bioremediation of heavy metals (Whiting et al. 2001). Upon exposure to heavy metal stress, rhizobacteria alter plant metabolism, due to which plants are able to withstand high concentrations of metals (Welbaum et al. 2004). The use of rhizobacteria in

phytoremediation has therefore recently gained some momentum (de Souza et al. 1999). The symbiotic effectiveness of bacteria-plant system for the restoration of polluted soil from chromium and cadmium contamination was studied by Sobariu et al. (2017) where they utilized rhizospheric *Azotobacter* bacteria and *Lepidium sativum* plant for completing this task. They observed that the ability of heavy metal tolerance by plant improved under symbiotic condition. Furthermore, bacterial consortia native to heavy metal-contaminated soil, consisting of *Bacillus mycoides* and *Micrococcus roseus*, were found effective for phytoextraction and phytostabilization of Cd (Malekzadeh et al. 2012). Bioremediation of zinc was mediated by rhizobacteria (*Bacillus megaterium* and *Pseudomonas aeruginosa*) isolated from weed (*Suaeda nudiflora*) growing in chemically polluted site (Jha et al. 2017).

Important genera of cadmium-resistant rhizobacteria reported from some food crops (wheat, maize, barley, mustard, mung bean, black gram, and pumpkin) are *Pseudomonas* spp., *Burkholderia* sp., *Flavobacterium* sp., and *Arthrobacter myso-rens* (Belimov and Dietz 2000; Ganesan 2008; Sinha and Mukherjee 2008; Kuffner et al. 2010; Xu et al. 2012; Saluja and Sharma 2014). Similarly, some arsenic-resistant gram-positive rhizobacteria are *Bacillus megaterium*, *Bacillus pumilus*, *Bacillus cereus*, *Arthrobacter globiformis*, and *Staphylococcus lentus*, while gram-negative rhizobacteria include *Rhizobium radiobacter*, *Rhizobium rhizogenes*, *Enterobacter asburiae*, *Agrobacterium radiobacter*, *Sphingomonas paucimobilis*, and *Pantoea* spp. (Wang et al. 2011; Titah et al. 2014; Lampis et al. 2015; Singh et al. 2015; Mesa et al. 2017). Rafique et al. (2015) reported some bacterial genera (*Bacillus*, *Pseudomonas*, and *Cronobacter*) capable of showing dual functions, i.e., simultaneously showing resistance for mercury, as well as capable of nitrogen fixation. Likewise, rhizobacteria capable of tolerating chromium are *Pseudomonas*, *Ochrobactrum*, *Mesorhizobium*, *Bacillus*, *Paenibacillus*, *Cellulosimicrobium*, and *Rhodococcus* (Faisal and Hasnain 2006; Trivedi et al. 2007; Chatterjee et al. 2009; Khan et al. 2012; Hemambika et al. 2013; Upadhyay et al. 2017).

### 10.3.2 Mechanisms of Heavy Metal Tolerance in Bacteria

Microbes are persistently able to survive in heavy metal-polluted environment by using a number of methods like biosorption, biomineralization, bioaccumulation, and biotransformation. Bioaccumulation is a process by which bacteria accumulate heavy metals in its cell which is influenced by various physical, chemical, and biological mechanisms operating inside its cell (Ayangbenro and Babalola 2017). Similarly, biosorption is defined as the passive uptake of metals by microbes (Malik 2004; Gadd 2009). Biomineralization is the process by which microbes form minerals. Likewise, biotransformation is another way of showing resistance toward heavy metals by microbes. It is the process of chemical alteration of chemicals such as nutrients, amino acids, toxins, and drugs by an organism. The two

important factors involved in the biotransformation of heavy metals in soil are pH and carbon sources. Biotransformations of heavy metals are demonstrated in algae, fungi, and prokaryotes which convert these metals into metal sulfides. However, being insoluble in nature, these metal sulfides have reduced bioavailability (Scarano and Morelli 2003; Ayyasamy and Lee 2012). Further, microbial biofilms have the ability of accumulating or sequestering heavy metals by producing EPS (exopolysaccharides) which bind with these metals (Teitzel and Parsek 2003; Meliani and Bensoltane 2016).

Due to the presence of anionic structures, microbes have a net negative charge on their surface. This negative charge enables them to bind with metal cations. Furthermore, the polarized groups of the bacterial cell wall or the capsule enable them to bind with metal ions (El-Helow et al. 2000). Binding of these metal ions to the cell wall is governed by several attractive forces like van der Waals forces, electrostatic interactions, covalent binding, and alterations in redox potential. De et al. (2008) reported that *Pseudomonas aeruginosa* contains cysteine-rich transport proteins located in their cell membrane which enabled them to adsorb exceptionally high amount of mercury, i.e., up to 400 mg Hg g<sup>-1</sup> dry cell mass. Microorganisms produce several organic and inorganic acids which help them in extracting metals from solid substrates.

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## 10.4 Our Lead

Since the last 20 years, our group is pursuing a lot of studies related to bioremediation of heavy metals. In the case of microbial bioremediation of arsenic, we observed that the presence of a similar mechanism of resistance in the two bacterial strains isolated from two different sources may be due to horizontal gene transfer of the arsenic gene *ars C* from soil to water system and vice versa which is an alarming situation for global concern (Saluja et al. 2011). Gupta et al. (2002) developed heavy metal-resistant mutants of phosphate-solubilizing *Pseudomonas* sp. Similarly, Tripathi et al. (2004) characterize siderophore-producing lead- and cadmium-resistant *Pseudomonas putida* KNP9 strain. Gupta et al. (2005) did an in situ characterization of mercury-resistant growth-promoting fluorescent *Pseudomonads*. However, we also characterize some other cadmium-resistant strains (Rani and Goel 2009; Kumar et al. 2019). Rani et al. (2008) reported some rhizobacteria responsible for the decline of copper toxicity in pigeon pea and soil system. Besides this, our group has also reviewed several studies related to rhizobacterial detoxification of heavy metals for crop improvement and has compiled them for readers of scientific communities to comprehend its knowledge in a simpler way (Rani and Goel 2009; Goel et al. 2017; Saluja et al. 2011; Khan et al. 2011).

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