Chapter 12
Role of Micro-organisms in Modulating Antioxidant Defence in Plants Exposed to Metal Toxicity

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Abstract Micro-organisms play diverse role rhizosphere where they interact and develop mutualistic associations. They stimulate plant growth by synthesising various metabolites and phytohormones like auxins (IAA), cytokinins and gibberellins etc. They also help to alleviate the oxidative stress induced by heavy metals by lowering the free radical formation and activation of different antioxidants and antioxidative enzymes (SOD, APOX, CAT, GR, POD, MDHAR, GPX etc.). Furthermore, they modulate the activity of ROS- scavenging pathways and maintain ROS homeostasis thereby, averts ROS- initiated inhibition of plant cellular processes and enhance their survival under metal stress. Moreover, they elevate redox state of plants by increasing the activities of ascorbate-glutathione recycling enzymes under metal stress. They also alter the levels of organic acids, phenols, flavonoids and siderophores which act as a part of metal detoxification and antioxidative defence system in metal-stressed plants. Plant- microbe symbiosis enables accumulation of stress-responsive phytohormones and trigger antioxidative defence responsive genes which enhance overall survival of plants under metal stress.

Keywords Oxidative damage · Reactive oxygen species · Antioxidative defense · Phenolic compounds · Siderophore production · Organic acid production

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12.1 Introduction

Among different abiotic stresses, the effect of heavy metal accumulation is known to be most hazardous factor in environment. Different heavy metals like Zn, Cd, Hg, As, Cu, Pb etc. are the interminable and bio accumulating elements which are highly toxic to living organisms and micro-organisms (Azevedo et al. 2012). These can enter the environment through natural or anthropogenic sources (Zawoznik et al. 2007). Metals can enter the plant roots via appoplastic or symplastic mechanisms which can further be translocated towards the shoot (Lux et al. 2011). They can lead to structural as well as biochemical changes like changing the redox state of the plant thereby inducing the oxidative stress and damaging membrane integrity (Gratao et al. 2009). Moreover, they also undergo physiological changes within the plants such as photosynthesis, mineral uptake and water relations (Gill et al. 2012).

In addition to this, they stimulate ROS production such as hydroxyl radical (OH·), superoxide radical ($O_2^{\bullet}$) and hydrogen peroxide (H_2O_2) (Benavides et al. 2005). ROS can stimulate lipid peroxidation by altering membrane lipids and affecting membrane permeability and fluidity (Tian et al. 2012). Accordingly, this causes ROS scavenging mechanisms to get accelerated. Plants possess different mechanisms to protect themselves against metal attack such as through the production of metal chelators and activation of different antioxidative defense mechanisms (Kopittke et al. 2010). Alternatively, some key enzymes involved in ROS detoxification such as superoxide dismutase (SOD), peroxidase (POD), glutathione peroxidase (GPX), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR) get activated (Gratao et al. 2008; Roychoudhury et al. 2012). Other non-enzymatic antioxidants like ascorbic acid, glutathione and secondary metabolites (flavonoids, organic acids, phenols and carotenoids) are also involved in ROS detoxification (Foyer and Noctor 2009).

Plant possess specific mechanism for detoxification and partitioning of metals between roots and shoots which could be directly or indirectly related to sequestration of metals between them (Mendoza-Cozatl et al. 2011). In this condition, they may establish symbiotic relationship with microbes from which they could be benefited by alleviation of the toxic metals along with the elevation of antioxidative responses of the plants (Garg and Chandel 2010; Sousa et al. 2012). These interactions could further be involved in enhancing plant productivities and their adaptive functions (Andrews et al. 2012). Microbial inoculations in metal stressed plants are proved to be involved in improving their growth and antioxidant defense system. According to Islam et al. (2014a, b), *Pseudomonas aeruginosa* stimulated antioxidant enzymatic activities of SOD, POD and CAT which further decreased MDA and H_2O_2 levels through ROS scavenging under Zn stressed wheat plants. Similar studies were found in *Solanum nigrum* under stress where *P. aeruginosa* (ZGKD5, ZGKD2) upregulated CAT, SOD, POD and APX levels and alleviated oxidative stress generated (Shi et al. 2016). Moreover, improved antioxidative defense system in wheat under Cu stress was reported by *Bacillus* inoculation through increased activities of SOD, POD, CAT, DHAR, APX by eliminating H_2O_2 and superoxide radicals in plants (Wang et al. 2013). Further, Madhaiyan et al. (2007) reported that plant growth promoting bacterial strains; *Methylobacterium oryzae* and *Burkholderia* sp. isolated from rice enhanced the growth of tomato plant by alleviating Cd stress. For instance, many microbial strains are also involved in modulating the activities of phenolic compounds and PAL (phenylalanine ammonia lyase) under metal stressed conditions (Mollavali et al. 2016). The addition of plant growth promoting bacteria to Cr stressed *Zea mays* L. induced phenolic contents within the plants. This combinational study showed that phenolic compounds act as metal chelators and elevated upon addition of microbial consortium (Islam et al. 2016a, b). Along with these important aspects, the indirect role of many other metabolites such as low molecular weight organic acids exuded by microbes have been elucidated in nutrient mobilization and metal solubilisation in rhizosphere (Rajkumar et al. 2012). The production of organic acids like oxalic acid, maleic acid, tartaric acid, succinic acid and formic acid by plant associated micro-organisms have gained much more attention in increasing metal solubilisation. Further, studies have been found in which they also increased the levels of enzymatic and non-enzymatic antioxidants under Cr treated *B. juncea* plants (Mahmud et al. 2017).

Recent studies have demonstrated the importance of arbuscular mycorrhizal fungi (AM fungi) in alleviating metal toxicity effects in plants (Andrade et al. 2010). The presence of AM fungi *Glomus vesiforme* in *Lonicera japonica* revealed the correlation between the intensity of mycozzhizal infection and increased stimulation of enzymatic activities of CAT, APX, GR and elimination of oxidative stress generated by Cd (Jiang et al. 2016). Furthermore, AM fungi also enhanced defensive pathways in plants by stimulation of antioxidants. Such type of studies were found by Bhaduri and Fulekar (2012) in which AM fungi elevated enzymatic activities in Cd treated *Ipomoea aquatica* plants. Moreover, reduced oxidative damage was observed by inoculation of *Funneliformis mosseae* in *Solanum nigrum* under Cd stress (Jiang et al. 2016a, b). In addition, *Glomusmossae* increased the stimulation of glutathione in response to Pb and Cd stress in *Cajanus cajan* L. The present chapter intends to increase our knowledge on the role of micro-organisms in modulating the antioxidative defense system in plants as well as metabolites leading to plant defence and heavy metal detoxification.

12.2 Heavy Metal Pollution

Rapid rise in world population rate lead to continued industrialization worldwide resulting in enormous environmental problems. Natural resources such as soil, water and air shows contamination of heavy metals, organic solvents, pesticides etc. (Prasad et al. 2010). In nature low concentration of heavy metals are present (Rodriguez Martin et al. 2013) via weathering of parent material of soil, volcanic eruptions, atmospheric deposition, mineralization, erosion etc. (Zhang et al. 2011) but this heavy metals proves fatal when its concentration rises from required amount by anthropogenic activities which include industrialization, mishandling of waste, agricultural chemicals (Zhao et al. 2008; Xu et al. 2014). There are many ways by which heavy metal enters the environment and once they are introduced, they travel great distances from their original source thus contaminating ecosystem far and wide (Oves et al. 2016) resulting in heavy metals deposition in soil which leads to nutrient deficiency and are likely to make land barren. Few reports of different sources of heavy metals are mentioned in Table 12.1. Urbanization and industrialization near coastal areas results in heavy metal pollution (Xu et al. 2016) affecting the flora and fauna of the habitat. Anthropogenic activities increase the frequency of deposition of heavy metals which in turn directly or indirectly affect flora and fauna of the habitat (Wang et al. 2015) and our environment (Sayadi 2014). Enhanced

Table 12.1 Different sources of heavy metals

Sources	Heavy metals deposition	References
Surface runoff from rain/snow	Mercury (Hg)	Kowalski et al. (2007)
Earth's crust	Cadmium (Cd), Hg, Lead (Pb), Arsenic (As)	Jarup (2003)
Volcanic emissions, forest fires, atmospheric deposition	Pb, Hg, Nickel (Ni)	Li et al. (2014)
Atmospheric aerosols, windblown dust	Pb, Hg, Ni	Kang et al. (2011)
Weathering of sedimentary rocks like sandstone and dolomite	Manganese (Mn), Chromium (Cr), As, Iron (Fe), Zinc (Zn)	Viers et al. (2007)
Geological minerals	As, Cr, Pb, Mn	Kibria (2014)
Ores/mineral disolution	Fe, Mn, Cr, Cd, Hg	Huffmeyer et al. (2009)
Sulfide mineral deposits	As	Nordstorm (2002)
Phosphate fertilizers	Cd	Jarup et al. (2003)
Pesticides	Hg, As, Cu, Zn	Arao et al. (2010)
Poultry waste, feed additives	As	Mukherjee et al. (2006)
Sewage, manures, sludge, limes	Cd	Yanqun et al. (2005)
Waste dumping	Cd, Pb, Cu	Zhuang et al. (2009)
Aerosols	As, Zn, Cd, Pb	Nagajyoti et al. (2010)
Fossial fuels combustion, chemical industries, electroplating.	Ni, As, Cr	Khodadoust et al. (2004)
Industrial waste pipes/additives	Cu	Mohod and Dhote (2013)
Sewage irrigation	Cd, Pb, Cr, Hg	Su (2014)
Industrial wastes	As	Tripathi et al. (2007)
Coal mining and steel processing industries	Zn	Greaney (2005)
Mining activities/smelting	Cr, Pb, Zn	Sumner (2000)

level of heavy metals like chromium, nickel, cadmium, arsenic, mercury, lead, copper, silver, zinc affect human health also by acting as neurotoxic, cytotoxic, mutagenic and carcinogenic agents (Ahmad et al. 2016a, b).

12.3 Metal Toxicity Effects on Plants

In response to metal toxicity, plethora of physiological and biochemical phenomena are altered (Villiers et al. 2011; Gill 2014). Reduction in growth is the primary visible symptom of metal stress (Sharma and Dubey 2007). Other common symptoms include chlorosis of leaves, change in water balance, decline in rate of seed germination and disruption of photosynthesis as shown in Fig. 12.1 (DalCorso et al. 2010; Kohli et al. 2013, 2017).

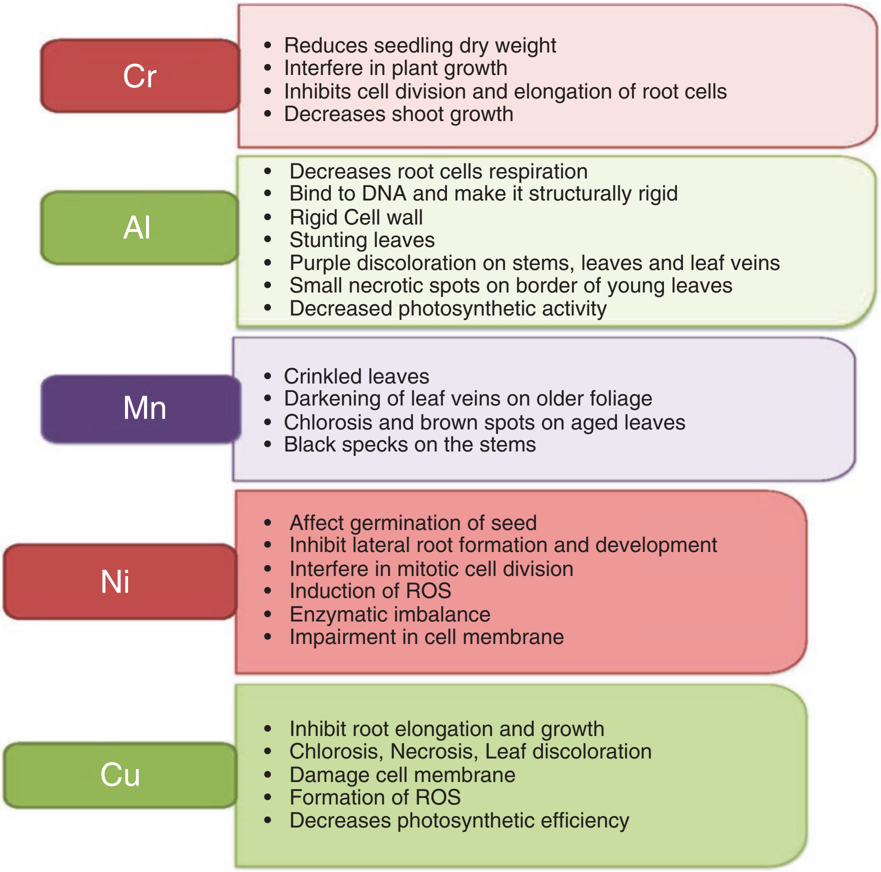

Fig. 12.1 Metal toxicity symptoms in plants

The severity of metal toxicity is attributed to the chemical composition and behaviour of metal in plants system. The toxicity of metal is dependent upon several factors including: (i) functional sites on protein moieties, (ii) disrupted enzyme functioning, (iii) elevation in synthesis of ROS and (iv) activation of fenton reaction (Halliwell 2006; Keunen et al. 2011). Plants have to maintain homeostasis between essential and hazardous concentration of metal ions in order to attain an ionic balance at cellular, tissue and organ level (Ovecka and Takac 2014). The early symptoms of metal toxicity in plants also include generation of Reactive Oxygen Species (ROS) resulting in oxidative stress (Maksymiec et al. 2007; Sharma and Dietz 2009). Oxidative stress is generally described as imbalance between generation of ROS and their scavenging by antioxidative defense components (Keunen et al. 2011). ROS are synthesized in all aerobic organisms and are one of the imperative signaling molecules in plant system (Foyer and Noctor 2005). The enhancement in ROS levels results in DNA damage, conformational changes in metabolites including protein, carbohydrates and lipids (Borsetti et al. 2005).

O_2 (molecular oxygen) in the atmosphere acts the final electron acceptor resulting in formation of ROS such as hydrogen peroxide (H_2O_2), hydroxyl radicle ($OH^{\cdot}$) and superoxide anion ($O_2^{\cdot -}$) in plants (Temple et al. 2005; Scandalios 2005). Most of the reactive $O_{2,}$ intermediates the oxidation of O_2 to singlet oxygen (Triantaphylides et al. 2008). The OH radicle have a non-selective reactivity whereas, O_2^- and H_2O_2 have specific reactivity towards biological macromolecules (D'Autreaux and Toledano 2007). Hydroxyl ions are the most reactive and harmful form of ROS. Another important ROS is nitric oxide (NO), which leads to formation of peroxynitrile radicals ($OONO^-$) by reacting with molecular O_2 (Gill and Tuteja 2010). Few other ROS includes peroxy, alkoxy and perhydroxyl radical etc. (Bhattacharjee 2005). The metal ions which are non-redox active in nature don't increase the production of ROS, although they inhibit activity of certain essential enzymes of the antioxidative defense system (Schutzendubel and Polle 2002). The highly oxidizing nature of ROS and its ubiquitous presence in peroxisomes, mitochondria and chloroplast indicates the predominant effect on photosynthesis and activation of antioxidative defense system (Seth et al. 2012). Figure 12.2 represents diagrammatic representation of heavy metal induced oxidative damage and repair.

12.4 Mechanism of Enhanced Antioxidative Defense by Microbes

Plant- microbe interactions have been widely employed in enhancing antioxidant defense systems in plants. They enhance resistance in plants towards abiotic stresses also known as IST (Induced Systemic Tolerance). They have been reported to induce genetic as well as metabolic potential in alleviating abiotic stress in plants. The modulating actions of different microorganisms in plant defense system by alteration of different antioxidative enzymes under abiotic stress have been studied in

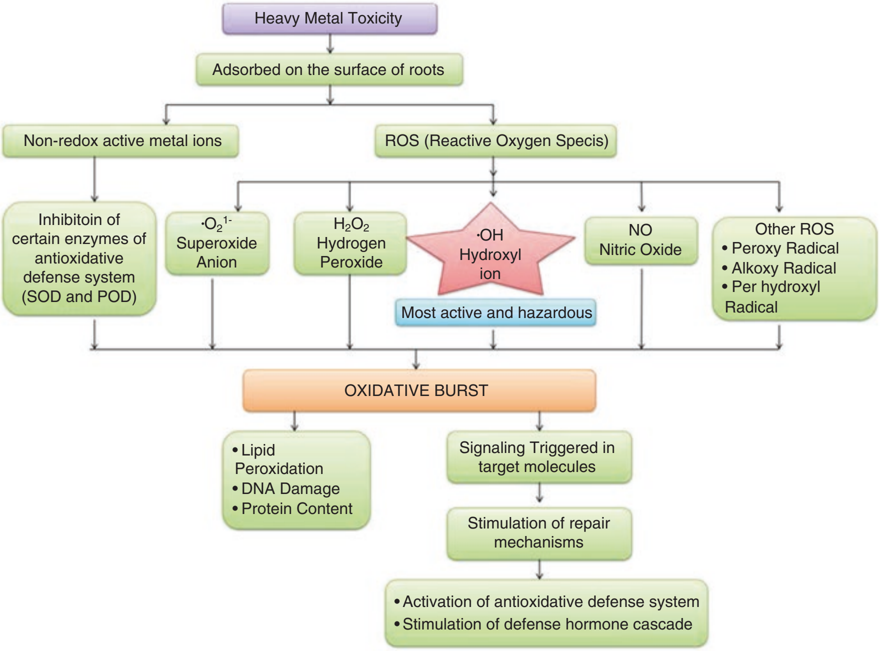

Fig. 12.2 Schematic representation of heavy metal induced oxidative damage and repair

recent past. However, the inoculation of different microorganisms in the rhizosphere effects the production of different metabolites in the root region as well as inside the plants. The effect of on some of the important metabolites during plant-microbe interaction has been discussed below:

12.4.1 Antioxidant Enzymes

Plant possesses a comprehensive and a complex system of antioxidative enzymes. This system of enzymes ameliorates toxic effect of wide array of stresses (Bano and Ashfaq 2013). The antioxidative enzymes include SOD, POD, APOX, GR, GPOX, GST, CAT, PPO, DHAR and MDHAR. The activities of several antioxidative enzymes are lowered in response to elevation in content of ROS by metal stress (Gill and Tuteja 2010). The enhancement in the metal toxicity results in elevation of levels of ROS consequently leading to disruption of metabolic pathways; cellular structures and functions are irreparably altered (Gratao et al. 2005). One of the most efficient methods for removal of toxic metals from soil includes use of microbes. Microbes play a critical role in remediation of heavy metals pollution from the soil and plant system. There exists a wide range of reports of microbes assisted modulation of antioxidative defense activities in pants under the effect of severe metal toxicity (Pandey et al. 2013; Ahmad et al. 2016a, b; Islam et al. 2016a, b). Few reports have been tabulated in Table 12.2.

Recent developments in the field of microbiology and environment management indicate use of microbes present in the rhizosphere or inoculation in the soil and further elucidate their crucial role in enhancing the antioxidative defense system of plants under heavy metal stress (Ma et al. 2010; Wang et al. 2011; Rajkumar et al. 2012). Bacterial inoculation of *Ricinus communis* in *Helianthus annus* plants exposed to Ni stress were reported to have enhanced activity of CAT and POD enzymes (Ma et al. 2010). Certain microbes have an ability to activate the antioxidative defense response endogenously in response to heavy metal stress. One such example is number of *Lactococcus* spp. and *Streptococcus* spp. which have been reported to have enhanced activities of Mn SOD (Poyart et al. 2002). Mycorrhizal fungi are an imperative class of heavy metal phytostabilizers. Plants which have an association with mycorrhizal fungi in their roots sequester huge range of metal pollutants in fungal hyphae and vesicles of the root system resulting in their immobilization as well as lowering their inhibitory actions. It was further suggested that, P and other essentials micronutrients uptake was also enhanced resulting in further enhancement in activities of antioxidative defense system including CAT, SOD and POD (Garg and Aggarwal 2011; Bano and Ashfaq 2013). A specific category of rhizobacteria includes plant growth promoting rhizobacteria (PGPR) have been reported to accelerate the phenomena of phytoremediation by modulating bioavailability of metal ions, oxidative damage, synthesis of phytohormones, siderophores interactions and binding of metal ions to the chelators (Ma et al. 2011a, b). A large number of studies suggest synthesis of several antioxidant compounds in response to endophytic fungi inoculation in plants resulting in enhancing the tolerance of plants (Malinowski and Belesky 2006; Yuan et al. 2010). Another report by Huang et al. (2007) suggested elevated antioxidative capacity and phenolic compounds of the host plants by endophytic fungi. This indicated that these endophytic fungi might possibly produce phenolic antioxidants.

12.4.2 Antioxidants

Plants possess different strategies in order to cope with different kinds of metals. The primary response of plants to metal stress is the generation of ROS such as O_2^-, H_2O_2 and HO (Gill et al. 2010; Yadav 2010). As a result, the plant cells respond defensively to oxidative damage by removing ROS and maintaining antioxidant defence compounds at levels that reflect ambient environmental conditions. The antioxidative system consists of both enzymatic and non-enzymatic antioxidants. Among non-enzymatic glutathione (GSH), ascorbic acid (ASA), tocopherol play major role in modulating defensive mechansims within plants (Mittler et al. 2004; Halliwell 2006; Scandalios 2005).

Table 12.2 Effect of microbes upon antioxidative enzyme system in metal stressed plants

Microorganisms	Plant species	Metals	Alteration of antioxidative defense system	References
Rhizobacterial isolates (SAN1, SAN2, SACC1 and SACC2)	*Triticum aesvitum* L.	Cd	GST, POD and CAT activities were elevated in metal stress plants inoculated with rhizobacterial strains	Hassan et al. (2016)
Glomus mosseae	*Solanum lycopersicum*	Cd	AMF mediated ROS scavenging through enhanced SOD, POD, APOX, GR and CAT levels	Hashem et al. (2016)
P. indica, Azotobacter chroococcum	*Triticum aesvitum* L.	Zn	Induced antioxidats activities as well as APOX and peroxidase activities stimulating ROS scavenging	Abadi and Sepehri (2016)
Enterobacter, *Lesfsonia*, *Klebsiella* and *Bacillus*	*Zea mays* L.	Cd	Activity of oxidases and catalase was lowered in response to bacterial inoculation. Membrane integrity and relative water content was not altered	Ahmad et al. (2016a, b)
Providencia vermicola	*Lens culinaris*	Cu	MDA content, H_2O_2 levels were significantly lowered whereas electrolyte leakage was remarkably elevated	Islam et al. (2016a, b)
Piriformospora indica and *Funelliformis mossae*	*Triticum aesvitum* L.	Cd	H_2O_2 levels were lowered by microbial treatment. CAT, APOX and GST enzymes activities were enhanced by inoculation by both fungal species and metal exposed plants	Shahabivand et al. (2016)
Sinorhizobium meliloti	*Medicago lupulina*	Cu	Inoculated plants showed stimulation in SOD, CAT, APOX and GR activities as an antioxidant defense mechanism	Kong et al. (2015)
Rhizobacterial isolates (ACC8, ACC5, AN8, AN12, *Azobacter*, RN5)	*Zea mays* L.	Pb	Plants which were inoculated with AN8 had maximum activities of CAT, POD and GST enzymes, although Pb content was lowered	Hassan et al. (2014)
Burkholderia (SNMS32, SCMS54)	*Solanum lycopersicum*	Cd	SOD, CAT, GR activities were enhanced by these strains, showing enhanced defensive mechanism against Cd	Dourado et al. (2013)

(continued)

Table 12.2 (continued)

Microorganisms	Plant species	Metals	Alteration of antioxidative defense system	References
Ochrobactrum sp. and *Bacillus* sp.	*Oryza sativa* L.	Cd, Pb and As	Bacterial inoculation resulted in decline in SOD enzyme activities as well as MDA content	Pandey et al. (2013)
Azospirrillum brasilense and Azotobacter chroococcum	*Triticum aestivum*	Pb	Improved enzymatic activities such as APOX, SOD, CAT via PGPR inoculation. Also improved membrane integrity, proline content and reduced MDA and H_2O_2 levels	Janmohammadi et al. (2013)
Glomes etunicatum	*Calopogium mucunoides*	Pb	Reduced levels of oxidative stress marker i.e. MDA. Lowered membrane damage caused by Pb due to fungal inoculants	de Souza et al. (2012)
Asbuscular mycorrhizal fungi (autochthonous micro-organism)	*Cajanus cajan*	Pb and Cd	CAT, POD, GR and SOD enzymes activities were elevated in response to AMF inoculation	Garg and Aggarwal (2012)
Glomus mosseae	*Cajanus cajan*	Cd and Zn	Upregulates antioxidant enzymatic activities such as SOD, POD, CAT, GR that alleviates heavy metal stress	Garg and Kaur (2013)
Agrobacterium radiobacter	*Populus deltoids* LHO5-17	As	Activation of antioxidative defense system. Significant enhancement in activities of SOD and CAT enzymes	Wang et al. (2011)
Psyhrobacter sp.	*Helianthus annus Ricinus communis*	Ni	CAT and POD enzyme activities were found to be enhanced by bacterial inoculation	Ma et al. (2010)
Pseudomonas aureofaciens and *Klebsiella oxytoca*	*Glycine max*	Cd	Enhanced glutathione *S*-transferase activities	Zaets et al. (2010)
Streptomyces acidiscabies E13	*Vigna unguiculata*	Al, Cu, Fe, Mn, Ni and U	MDA levels was observed to be lowered in siderophore supplemented plants when compared to metal treated plants	Dimkpa et al. (2009b)
Rhizobium sp. (RP 5)	*Piscum sativum*	Ni and Zn	Activity of GR enzyme in roots was enhanced. Also levels of H_2O_2 was lowered by activation of ascorbate-glutathione cycle	Wang et al. (2011)
Glomes etunicatum	*Lactuca sativa*	Zn	GPOX and APOX activities were drastically enhanced in response to AMF inoculation	Farshian and Malekzadeh (2007)

12.4.2.1 Glutathione

Glutathione (GSH; γ-glu-cys-gly) is the main source of non-protein thiols and are biochemically active in plants against different types of stresses. It plays a central role as chelating agent, antioxidant and signalling component and exists in both reduced (GSH) and oxidised (GSSG) forms (Jozefczak et al. 2012). Non enzymatic GSH is the first defence pathway in plants under stressed conditions. GSH after donating electron to ROS gets converted to GSSG and can be regenerated by action of GR at the expense of NADPH. In healthy cells more than 90% of the total GSH pool is in its reduced form but after metal treatment reduced GSH concentration is decreased (Mittler et al. 2004). Oxidation of several antioxidant pathways are interconnected with GSH. ASA-GSH cycle is second defence pathway in which AsA and GSH are oxidized and reduced to permit AsA peroxidise to neutralize H_2O_2 to H_2O as shown in Fig. 12.3 (Jozefczak et al. 2012).

Moreover, GSH quenches ROS generated in plants under heavy metal exposure. Along with this, it also enables phytochelatin synthesis in plants (Yadav 2010). It has been reported that GSH synthesis was enhanced in *Arabidopsis* plants under Cd and Ni stress (Freeman et al. 2004). Studies have been reported in which GSH forms complexes with metals and enables their transport across membranes. In this mechanism, AtATM3 plays important role, that mediates GSH-Cd transport along with mitochondrial membranes in *Arabidopsis thaliana* (Kim et al. 2006). The interaction between metal hyperaccumulator plants and plant growth promoting bacteria is an emerging technology in heavy metal accumulation and increasing the antioxidative defence system within them (Ma et al. 2016; Xun et al. 2015). Micro-organisms modulate the levels of antioxidants within plants under heavy metal stress conditions (Ahmad et al. 2016). Mycorrhizal fungi in association with the plants undergo sequestration of wide range of heavy metals in fungal hyphae and roots of the plants. This limits their inhibitory effects on growth as well as undergoes immobilization of metal ions (Garg and Kaur 2013). It was further suggested that GSH levels have

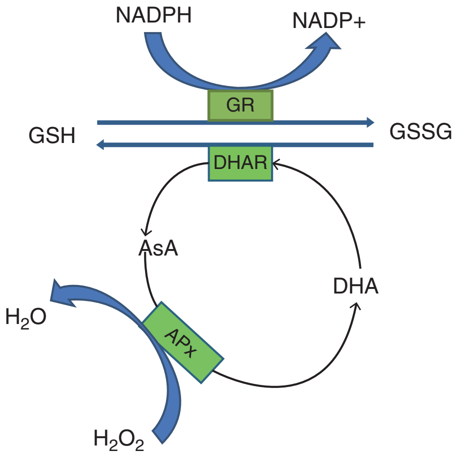

Fig. 12.3 Oxidised and reduced ASA-GSG forms resulting in conversion of H_2O_2 to H_2O

been increased in *Cjanus cajan* upon mycorrhizal inoculation of *Glomus mosseae* under Cd stress which reduced the oxidative stress generated in the plant (Garg and Kaur 2013). Similar reports have been found in which this AM fungi stimulated GSH levels in the *Cjanus cajan* plant under Zn stress thereby upregulating the defensive mechanism of the plant (Garg and Kaur 2013).

Various reports showing the role of microbes in modulating glutathione activity in plants are summarized in Table 12.3:

12.4.2.2 Ascorbic Acid

AsA is one of the universal non enzymatic antioxidant for detoxification of ROS (Akram et al. 2017). Ascorbate is physiological active form of AsA that is a resonance stabilized anionic form formed by deprotonation of OH group at C3 carbon and it is water soluble antioxidant within living system (Smirnoff 2000a, b). ASA is involved in activation of antioxidant enzymes and also act as a source of electron for activation of APXs. For eg: APXs are heme containing enzymes that dismutase H_2O_2 to H_2O and molecular oxygen (Mittler et al. 2004; Van Doorn and Ketsa 2014). Ascorbate is significantly involved in the scavenging of $O_{2,}$ OH radicals and reduces H_2O_2 to H_2O respectively in plants exposed to metal toxicities. They are localised in cellular compartments such as chloroplasts and at optimal concentrations enables plant's defence against metal toxicities (Ray et al. 2016; Fuentes et al. 2016). They are not only restricted to chloroplasts but also plays a crucial role in ROS scavenging in cytosol, mitochondria and peroxisomes (Mittler et al. 2004). Furthermore, soil microorganisms enhance plant growth and quality in mutualistic association by alteration in ascorbic acid levels. They also increase biotic and abiotic stress tolerance of host plant by increasing or decreasing the activity of these antioxidants (Mollavali et al. 2016). Studies were reported in which endophytic bacteria stimulated ascorbic acid content along with glutathione by acting as redox buffers (Ray et al. 2016). It was further observed that ASA levels were increased in the maize plants under Cr stress, which upon PGPB incoulation was further elevated upto certain levels. It signifies that AsA promoted plant growth and minimised H_2O_2 and MDA content, showing the protective role against oxidative stress tolerance upon bacterial inoculations (Islam et al. 2016). Moreover, bacterial inoculation of *Bacillus licheniformis* and *Pseudomonas flurescens* in grape vine exposed to As stress stimulated AsA levels (Pinter et al. 2017). Various reports of microbes modulating ascorbic acid in plants under heavy metal stress are given below in Table 12.4.

12.4.3 Phenolic Compounds

Metal toxicity results in oxidative stress which interferes with physiological activities of plants including photosynthesis, respiration and inhibition of imperative enzymes (Shanker et al. 2005; Singh et al. 2013). Various secondary metabolites of

Table 12.3 Effect of microbes on glutathione levels in metal stressed plants

Microbes	Plant species	Metals	Effect on glutathione activity	References
Ectomycorrhiza *(P. tinctrius)* Endomycorrhiza *(E. colombiana, G. clarum)* Beneficial bacteria (*B. licheniformis, B. subtilis, B. thuringiensis*	*Acacia saligna* and *Eucalptus camaldulensis*	As, Cd, Pb, Zn	Induced GSH levels in plants	Guarino and Sciarrillo (2017)
Funneliformis mosseae	*Solanum nigrum*	Cd	Enhanced antioxidant activities and reduced MDA levels relieved oxidative damage from Cd-treated plants	Jiang et al. (2016a, b)
Exophiala pisciphila	*Zea mays*	Cd	Enhanced total ROS scavenging by increased GSH content	Wang et al. (2016)
Streptomyces pactum (Act 12)	*Amaranthus hypochondriascus*	Cd	Stimulated levels of GSH in leaves with microbial inoculations	Cao et al. (2016)
AM fungi *(Glomus versiforme, Rhizopus intraradices)*	*Lonicera japonica*	Cd	Reduced glutathione levels in inoculated plants	Jiang et al. (2016a, b)
Endophytic bacteria *Serratia* sp. RSC-14	*Solanum nigrum*	Cd	GSH contents increased significantly in plants exposed to Cd but more pronounced in bacterial inoculated plants	Khan et al. (2015)
Arbuscular mycorrrhizae	*Eucalyptus camaldulensis*	As, Cd, Zn, Pb, Ti, Hg	Formation of HM-chelating complexes by glutathione deriving phytochelatins in the presence of AM/PGPR	Guarino and Sciarrillo (2017)
			Increased glutathione synthesis	
Endophyte Serratia nematodiphila LREO7	*Solanum nigrum*	Cd	Exhibited higher GSH activities in metal stressed plants which further enhanced upon microbial inoculations	Wan et al. (2012)
Glomus mosseae	*Cjanus cajan*	Cd	Enhanced glutathione levels alleviating oxidative stress	Garg and Kaur (2013)

(continued)

Table 12.3 (continued)

Microbes	Plant species	Metals	Effect on glutathione activity	References
Glomus mosseae	*Cjanus cajan*	Zn	AM fungi-mediated elevated glutathione contents upregulating antioxidative defense system of the plant	Garg and Kaur (2013)
Glomus mosseae	*Cjanus cajan*	Cd Pb	GSH levels increased acting as potential biomarkers for defensive system and metal detoxification	Garg and Aggarwal (2011)
Paecilomyces lilacinus NH1	*Solanum nigrum*	Cd	Increased GSH upon fungal inoculation promoting detoxification of ROS as well as oxidative stress	Gao et al. (2010)
Rhizobium sp. RP5	Pea	Zn and Ni	Stimulated glutathione activities in roots and nodules in presence of bioinoculant which enables detoxification of H_2O_2 via ascorbate glutathione cycle	Khan et al. (2009)

plants like flavonoids, proline, total phenols aid in detoxification of heavy metals (Gill and Tuteja 2010). Phenolic compounds (i.e. phenols, phenylopropanoids, phenolic acids and flavonoids) categorized as low molecular weight antioxidants can directly sacavange free radicals. These compounds are an electro-donating agent which act as antioxidants, provides colour and contribute to overall health of plants.

12.4.3.1 Phenols

Phenols are one of the major secondary metabolites found in plants. Phenols also act as metal chelators under heavy metal stress and their metabolism is induced during metal stress depending upon their composition. They are mostly oxidized through H_2O_2 scavenging or Asc-POX system (Michalak 2006). The content of plant phenols are mostly enhanced in response to heavy metal stress which is also confirmed with the studies of Marquez-Garcia et al. (2012) for *Erica andevalensis* and Ahmad et al. (2015a, b) in *Cannabis sativa*. The interaction of plants with microbes is considered as important component of soil ecosystem and this interaction is beneficial for plants in coping up various forms of stresses. The applications of these microorganisms have major impact in tolerating and alleviating heavy metal stress. Increase in the content of total phenolics and activity of phenylalanine

Table 12.4 Effect of microbes on AsA content under metal stressed plants

Microbe	Plant species	Metals	Effect on ascorbic acid activity	References
B.adusta *Mortierella* sp.	*S. lycopersicum*	Cu, As, Pb and Zn	Induced AsA levels in plants co-inoculated with the fungal consortium	Fuentes et al. (2016)
AM fungi *(Rhizophagus intraradices)*	*Lonicera japonica*	Cd	Activated AsA-GSH pathway reducing H_2O_2 and MDA levels	Jiang et al. (2016a, b)
Proteus mirabilis	*Zea mays*	Cr	AsA concentration is significantly higher in plants inoculated with PGPB	Islam et al. (2016a, b)
R. irregularis	*S. lycopersicum*	Various heavy metals	Higher AsA levels in plants colonized by *R. irregularis*	Fuentes et al. (2016)
Azotobacter croococcum, *P. indica*	*Triticum aestivum*	Zn	Bacteria mediated antioxidants levels (AsA) alleviating oxidative stress	Abadi and Sepehri (2016)
AM fungi (*Glomus versiforme)*	*Lonicera japonica*	Cd	Elevated levels of AsA lowering H_2O_2 content via ascorbate-glutathione pathway	Jiang et al. (2016a, b)
Pseudomonas aeruginosa	*Triticum aestivum*	Zn	Bacteria enhanced non-enzymatic antioxidants (AsA) as well as decreased MDA and H_2O_2 content through ROS scavenging by antioxidants	Islam et al. (2014a)
Proteus mirabilis	*Zea mays*	Zn	Inoculated plants enhanced ascorbic acid levels, providing upregulation of defense system	Islam et al. (2014b)

ammonia-lyase (PAL) was observed under mycorrhizal symbiosis with plants (Nell et al. 2009; Mollavali et al. 2016). The effect of application of microorganisms on plant phenolics has been listed in the Table 12.5.

12.4.3.2 Flavonoids

Flavonoids are organic compounds widely distributed in plants as secondary metabolites. More than 9000 naturally occurring flavonoids are known and plays wide range of role, few of which are shown in Fig. 12.4 (Buer et al. 2010). One of the major role of flavonoids are its antioxidative properties which is due to its structure i.e. conjugated double bonds and functional group in the rings (Seyoum et al. 2006).

It provides defensive role in plants by protecting them from biotic and abiotic stresses. It reduces the production of reactive Oxygen Species (ROS) while quenching its action by inhibiting the ROS generating enzymes, free radical quenching in lipid peroxidation and regeneration of other antioxidants (Arora et al. 2000; Higdon and Frei 2003). Flavonoids are mostly secreted from root tips and root hair zone as they are the target sites for symbiotic relationship between plant and bacteria

Table 12.5 Effect of microbes on phenols in metal stressed plants

Microorganism	Plant species	Heavy metal	Effect on phenolic compounds (phenols)	References
Paecilomyces formosus LH10	*Glycine max* L.	Ni	Increased activity of phenolic acid	Bilal et al. (2017)
Bradyrhizobia (R) and Arbuscular mycorrhiza (AM)	*Glycine max* L.	Zn	Enhanced content of total polyphenols under dual treatment	Ibiang et al. (2017)
Plant growth promoting bacteria (T2Cr and CrP450)	Zea mays L.	Cr	Induced total phenolic content	Islam et al. (2016a, b)
Providencia vermicola	*Lens culinaris*	Cu	Decline in the content of total phenolics	Islam et al. (2016a, b)
Bradyrhizobia and arbuscular mycorrhizal fungi	*Glycine max* L.	Zn	Stimulation in total polyphenol content	Ibiang et al. (2017)
Claroideoglomus claroideum, Funneliformis mosseae	*Calendula officinalis*	Cd Pb	Upregulation of antioxidant capacity by accumulation of phenolic compounds	Hristozkova et al. (2016)
Arbuscular mycorrhizal fungi	*Solanum lycopersicum* L.	Cd	Enhanced content of phenols	Hashem et al. (2016)
Cellulosimicrobium funkei	*Phaseolus vulgaris*	Cr (IV)	Elevated levels of phenolic acid	Karthik et al. (2016)
Enterobacter asburiae KE17	*Glycine max* L.	Cu, Zn	Reduced total polyphenol content	Kang et al. (2015)
Arbuscular fungi (*Glomus versiforme*)	*Poncirus trifoliate*	Fe	Up regulated activities of phenylalanine ammonia lyase (PAL) and *pal1* gene expression	Li et al. (2015)
			AMF mediated alleviation of Fe stress by enhancing the levels of ferulic acid, salicylic acid, gallic acid, phlorizin etc	

(continued)

Table 12.5 (continued)

Microorganism	Plant species	Heavy metal	Effect on phenolic compounds (phenols)	References
Pseudomonas aeruginosa	*Triticum aestivum*	Zn	Enhanced total phenolic compounds (TPC)	Islam et al. (2014a, b)
			Improved plant growth while decreased H_2O_2, MDA content and protection against oxidative stress	
Rhizobium tibeticum	*Trigonella foenumgraecum*	Co	Increased PAL activities that further enhanced polyphenol biosynthesis in inoculated plants	Abd-Alla et al. (2014a)
Rhizobium tibeticum	*Trigonella foenumgraecum*	Ni	Promoted phenylalanine ammonia lyase (PAL) activities that increased the biosynthesis of polyphenols	Abd-Alla et al. (2014b)
Bacterial consortium (*Pseudomonas* sp. IMBG163,IMBG164, *Paenibacillus* sp. IMBG156, *Klebsiella oxytoca* IMBG26, *Pantoea agglomerans* IMBG56, *Bradyrhizobium japonicum* IMBG172, and *Stenotrophomonas maltophilia* IMBG147)	*Glycine max* L.	Cd, Cu, Zn	Enhanced content of soluble phenolics	Zaets et al. (2010)

Fig. 12.5 (Abdel-Latief et al. 2012). It protects the plant's photosynthetic machinery from harmful UV-radiations as well as aids plant- bacterial symbiotic relationship. It also plays critical role as specific transmitters legumes secrete luteolin and chrysin as flavonoid which acts as signal for bacteria population to initiate symbiosis (Mierziak et al. 2014). It was further reported that alfalfa roots release large amount of flavonoids due to reduction in nitrogen content. Moreover, in response to alfalfa derived flavonoids, *Rhizobium meliloti* shows chemotaxis towards luteolin, 4′,7-dihydroxyflavone and 4′,7-dihydroxyflavanone and the chalcone 4,4′-dihydrochalcone (Cohen et al. 2001).

Fig. 12.4 Role of flavonoids in plants

Although there are many studies in which micro-organisms enhance plants defensive potential by modulating flavonoid levels in metal stressed plants (Li et al. 2015a, b). It was suggested that independent treatment of PGPB and SA on Cr stressed *Zea mays* L. plant led to up regulation of phenolic compounds which act as antioxidant and chelator by enhancing metabolic activity of plants (Islam et al. 2016). Furthermore, PGPB and SA combination enhances plant flavonoid content under metal stressed conditions which might be due to the exudation of siderophores or root-microbe interactions (Islam et al. 2009). Similar results have been found by various researchers on phenolic compounds with PGPB inoculation (Saravanakumar et al. 2007; Li et al. 2015). Various reports showing the effect of micro-organisms on phenolic compounds (flavonoids) in metal stressed plants are given below in Table 12.6.

Table 12.6 Effect of microbes on flavoinoid content in metal stressed plants

Microorganism	Plant	Heavy metal	Effect on phenolic compounds (flavonoids)	References
Claroideoglomus claroideum, Funneliformis mosseae	*Calendula officinalis*	Cd	Enhanced antioxidant capacity by accumulation of secondary metabolites i.e. flavonoids and total phenols	Hristozkova et al. (2016)
Proteus mirabilis	*Zea mays*	Cr	Enhanced flavonoid content by PGPR inoculation	Islam et al. (2016a, b)
Claroideoglomus claroideum, Funneliformis mosseae	*Calendula officinalis*	Pb	Rise in flavonoid levels exhibiting antioxidant defense	Hristozkova et al. (2016)
Calendula officinalis L.	AM fungi	Cd	Accumulation of total petal flavonoids that improved plant growth and yield	Tabrizi et al. (2015)
Rhizobium tibeticum	*Trigonella foenumgraecum*	Co	Increased total flavonoid contents along with nodulation, glutamine synthase (GS) and nitrogenase activity	Abd-Alla et al. (2014a)
Rhizobium tibeticum	*Trigonella foenumgraecum*	Ni	Total flavonoid content induced subsequently after bacterial inoculation	Abd-Alla et al. (2014b)
AM fungi (*Glomus irregularis)*	*Medicago truncatula*	Cd	Cd-induced isoflavonoids and their derivatives such as formononetum, malonylonoxin, medicarpin and coumestrol accumulation in plant roots that was further reduced by AM fungal colonization	Aloui et al. (2012)
AM fungi	*Juniperus procera*	Pb Zn	AM-colonization enhanced flavonoid levels in leaves	Ghamdil et al. (2012)
AM fungi *Rhizophagus irregularis*	*Medicago truncatula*	Cd	Accumulation of isoflavonoids through AM colonization	Aloui et al. (2012)
AM fungi	*Juniperus procera*	Cd	Microbial- mediated stimulated flavonoids such as quercetin-3-glucoside, quercetin, rutin, quercitin-3- rhamnoside in metal exposed plants	Ghamdil et al. (2012)

12.5 Indirect Role of Siderophores in Enhancing Antioxidative Defence and Heavy Metal Detoxification in Plants

Siderophores are compounds having low molecular mass (400–1000 Da) but high chelation capacity for iron as well as with other metals like Al, Cd Cu, Pb and Zn etc. (Schalk et al. 2011). They are produced by rhizospheric microorganisms in

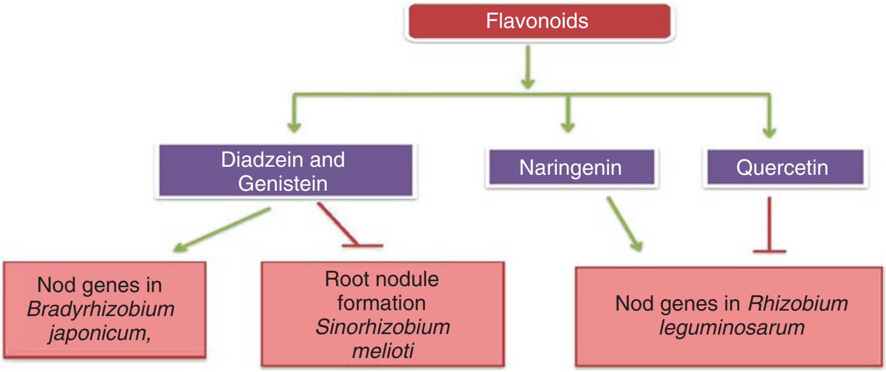

Fig. 12.5 Role of flavonoids in nodulation (Denarie et al. 1996; Cooper 2004; Reddy et al. 2007)

response to iron deficiency. The structural group of siderophores are classified on the basis of their chemical composition depending upon the moieties which donate oxygen ligand for complexation with Fe(III) i.e. α-hydroxy-) carboxylates, hydroxamates and catecholates. Siderophores act as solublizing agents and convert the unavailable heavy metal into soluble forms by forming chelation complexes which are sought to have important role in heavy metal phytoextraction (Braud et al. 2009b; Dimkpa et al. 2009a, b; Rajkumar et al. 2010). Enhanced uptake of Cr, Pb and Cd due to production of siderophores by *Pseudomonas aeruginosa* and *Streptomyces tendae* has been reported by Braud et al. (2009a, b) and Dimkpa et al. (2009a, b, c) respectively. Improved phytoextraction in plants by siderophores secreting microorganisms suggests their role in improved uptake by inoculating plants with siderophores. However, antagonistic reports of decreased metal uptake in presence of microbial siderophores have also been reported by Sinha and Mukherjee (2008) in *Cucurbita pepo* and *Brassica juncea* and Tank and Saraf (2009) in chickpea. However, the uptake of metal depends upon metal availability, type of plant and translocation of metal to shoots. Increased uptake of heavy metal with siderophore complexes induces oxidative stress in plants due to ROS production (Rajkumar et al. 2010). The siderophore producing microorganisms modulate the heavy metal induced oxidative stress by enhancing the antioxidative defense system of plants. These microorganisms also inhibit the IAA (indole-3-acetic acid) caused oxidative degradation in plants and increase the activity of enzymes like POD (peroxidase), SOD (superoxide dismutase) and carotenoids which have protective role against stress (Dimkpa et al. 2009a, b, c; Sharma and Johri 2003). The enhanced activity of enzymes by siderophores helps to overcome the detrimental effect on plant biomolecules as shown in the Fig. 12.6. The effect of siderophores producing bacteria on growth of mycelium and Pb and Cd uptake as well as antioxidant system was studied by Cao et al. (2012) in *Oudemansiella radicata.* Results demonstrated that siderophore exposure enhances the growth and Cd (by 26.5% in *Bacillus* sp.) and Pb accumulation (by 158.9%) in comparison to controls. Decrease in oxidative damage and reduced activity of enzymes like SOD and POD was

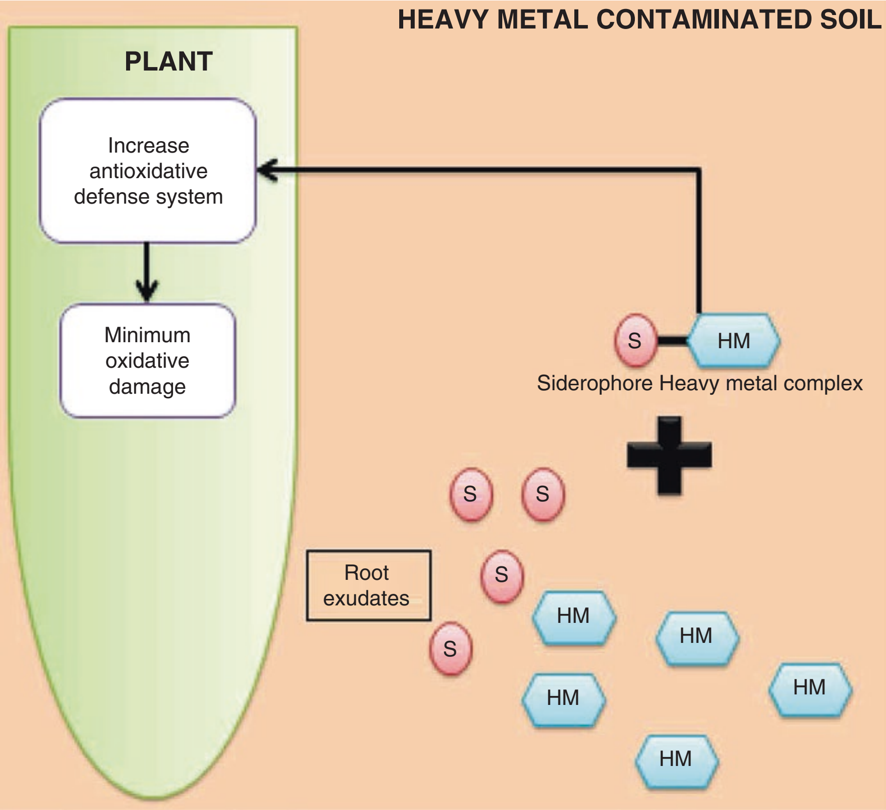

Fig. 12.6 Schemetic representation of siderophore effect on plant antioxidative defense system

observed which shows the role of siderophores in declining the toxicity of heavy metals.

12.6 Indirect Role of Organic Acids in Enhancing Antioxidative Defence and Heavy Metal Detoxification in Plants

Organic acids are CHO comprising compounds distinguished by the occurrence of one or more carboxyl groups with a maximum molecular weight (300 Da) (Jones 1998). In soil, they can bind to metal ions through complexation reaction. The stability of the ligand: metal complexes is reliant on various factors: (a) the nature of organic acids which includes number of carboxylic groups and their position, (b) the binding form of the heavy metals, (c) pH of soil (Jones 1998; Ryan et al. 2001). They might cause the acidification of the rhizosphere by decline pH and then mobilization of insoluble heavy metal chelates in soil and led to enhance their bioavailability (Seshadri et al. 2015). Organic acids are considered as agents of phytoremediation and also act as protectants to enhance tolerance against stress. Maleic acid (MA) increased the content of non-enzymatic antioxidants (AsA, GSH) and the activities of enzymatic antioxidants such as SOD, CAT, APOX, MDHAR, DHAR, GR, GPOX under Cr stress in *Brassica juncea* plants which further enhanced Cr uptake in the roots, but it slightly decreased the translocation of Cr from roots to shoots at lower concentration of Cr and considerably at higher concentration. MA decreased oxidative damage caused by Cr and improved the chlorophyll content, water status, growth and biomass of the plants (Mahmud et al. 2017).

Over last decades, the low molecular weight organic acids (LMWOAs) exuded by plant-associated microorganisms have gained much attention due to their suggested role in mobilization of mineral nutrients and solubility of heavy metal in the rhizosphere (Rajkumar et al. 2012).

Organic acids produced by plant-associated microorganisms play a significant role in the complexation of toxic and important ions and enhance their mobilization for plant uptake. Moreover, the role of organic acids in antioxidative defense system has been elucidated. It was investigated that, antioxidative defense system of *Solanum nigrum* L. was improved by supplementation of *Paecilomyces lilacinus* NH1 and citric acid. It promoted the plant growth under Cd stress and significantly alleviated the oxidative stress experienced by the plant (Gao et al. 2010). It was also reported that citric acid has the ability to enhance heavy metal accumulation in *Solanum nigrum* L. by enhancing antioxidative activities. It also enhanced chelation of heavy metals and stimulated antioxidant defense system in plants under Cd and Pb treatments (Gao et al. 2012). It was observed that enhanced antioxidative defense in plants was due to the expression of defense related enzymes or specific proteins (Gao et al. 2012). The further study conducted by Saravanan et al. (2007) reported that production of a gluconic acid derivative and 5-ketogluconic acid by *Gluconacetobacter diazotrophicus* strains further assisted in the solubilization of Zn compounds (ZnO, $ZnCO_3$ or $Zn_3(PO_4)_2$). It has been demonstrated that *P. aeruginosa* (CMG 823) had the potential to solubilize large quantity of ZnO and $Zn_3(PO_4)_2$) that was dependent on production of 2-gluconic acid (Fasim et al. 2002). Soil inoculated with Cd/Zn resistant bacteria showed enhanced water soluble Cd and Zn levels in *Sedum alfredii* in comparison to uninoculated soil. Increased mobility of heavy metals might be due to enhanced production of organic acids such as acetic acid, formic acid, tartaric acid, oxalic acid and succinic acid (Li et al. 2010). *Burkholderia caribensis* considerably mobilized P and Fe from phosphorous iron ore because of production of gluconic acid along with exopolysaccharides production which lead to the formation of biofilms that help in the mobilization of P and Fe from the ore (Delvasto et al. 2009). A study conducted by Wani et al. (2007) demonstrated that metal resistant *Bacillus* strains (PSB 1, PSB 7, and PSB 10) were taken for the mobilization of Pb and Zn and it was found that PSB1 strain had potential to mobilize large quantity of Pb and Zn. Mycorrhizal fungi can also release organic acids into the rihzosphere for the mobilization of heavy metals by forming complexes which further acidify the rhizosphere. It has been reported that ericoid mycorrhizal fungi *Oidiodendron maius* increased mobility of Zn from insoluble Zno and $Zn_3(PO_4)_2$ through secretion of Zn chelating malic and citric acid (Martino et al. 2003). Organic acids produced by fungi *Beauveria caledonica* aided in the solubilization of $Zn_3(PO_4)_2$ and pyromorphite via acidolysis (protonation) reaction (Fomina et al. 2005). Studies revealed that organic acids secreted by plant associated microorganisms assisted in plant root absorption of metal ions such as Cu (Chen et al. 2005), Pb (Sheng et al. 2008) and Cd and Zn (Li et al. 2010). *Pseudomonas fluorescens* G10 and *Microbacterium* sp. G16 (metal resistant endophytic bacteria) were reported to increase the accumulation of Pb in rape through secretion of organic acids (Sheng et al. 2008). *Aspergillus niger* was capable of mobilize large quantity of Pb and P from pyromorphite with the help of organic acid produced by this fungi. In addition, *A. niger* significantly increased uptake of Pb and P in *Lolium perenne* (Sayer et al. 1999). It was revealed that organic acids producing microorganisms have the capacity to improve the phytoextraction technique in metal polluted soils.

Organic acids have been suggested to play an important role in the mechanisms associated with uptake of heavy metals by roots (Han et al. 2006; Panfili et al. 2009). In maize, organic acids such as acetic and malic acids promoted Cd uptake by roots and demonstrated that the organic acid with low stability constant was capable to increase large quantity of Cd accumulation (Han et al. 2006). Maize roots were proficient to detach Cd from Cd-organic acid complex via root surface mediated process and thus led to enhanced Cd uptake. It has been showed that free Cd ions were more easily accessible to maize roots as compared to intact Cd-organic ligand complexes. Similarly, citric acid improved Cd uptake and its distribution among roots of durum wheat (Panfili et al. 2009). Complex formation between metal-organic acid indirectly participate in metal uptake by dissociation of metal from metal-organic acid complexes within diffusion layer or at surface of roots which led to enhance the level of free metal ions (Han et al. 2006; Panfili et al. 2009).

Various plant-associated microbes have the capability to produce organic acids and lead to mobilization of toxic and essential ions (Fomina et al. 2005; Martino et al. 2003; Uroz et al. 2009), a significant question that has yet to be satisfactorily resolved is either they act as sources or sinks of organic acids in the soil. In-vitro studies with soil microbes have elucidated to some level, in which the concentration of organic acid influx is directly normalized by the external concentration (Jones et al. 1996). Soil properties of rhizosphere (sorption, biodegradation, buffering capacity and metal complexation) may change the organic acids profiling, making complex to predict the behaviour of organic acids (Jones et al. 2003; Rajkumar et al. 2012; Mahmud et al. 2017). A comprehensive description of the factors those manage the fate and organic acids behavior in soil such as concentrations requisite for mobilization of metals, efficiency of heavy metal/nutrient-mobilization, biodegradation and sorption to the soil's solid phase are keys to recognize the exact mechanisms of organic acids produced by microbes in the metal polluted rhizosphere soils. The exact quantification of organic acids in soils and the full sequencing of organic acid producing microorganisms together with biomarker tools like green fluorescent protein-based biosensors, will help to understand the dynamics of transport of organic acid between rhizosphere soils, plants and microbes.

12.7 Conclusion

Micro-organisms associated with plants have the ability to aid the plant growth and metal immobilization or mobilization. Studies have indicated that they exhibit resistance against different heavy metals and protect the plants against adverse effects. Using microbial consortia, different activities of the plants like nodulation and anti-oxidative capacities are enhanced by reducing oxidative stress. In addition they also promoted heavy metal detoxification within plants by enhancing metal accumulation, translocation and phytoextraction. Further, most of the studies of microbes modulated the secondary metabolites produced within the plants and enhanced their antioxidative defense system. They have the potential to activate the plant enzymatic and non-enzymatic antioxidant system. Moreover, among secondary metabolites, phenolic compounds which aid metal detoxification and directly scavenge free radicals have also been modulated via microbial colonization. Apart from this, micro-organisms producing siderophores and organic acids under metal stressed conditions promoted plant growth by decreasing oxidative stress.

Such studies may enable us to manipulate microbes in improving plant's performance under metal stressed conditions and utilizing their potential in heavy metal detoxification. We anticipate that exploration of microbial consortium (both bacteria and fungi) possessing plant growth promoting features and their inoculation with hyperaccumulator plants would yield better results for enhancing their anti-oxidative potential as well as metal detoxification.

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