Nanotechnology in the Life Sciences

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Abdulaziz A. Al-Khedhairy *Editors*

Cellular and Molecular Phytotoxicity of Heavy Metals

Springer

Nanotechnology in the Life Sciences

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Nano and biotechnology are two of the 21st century's most promising technologies. Nanotechnology is demarcated as the design, development, and application of materials and devices whose least functional make up is on a nanometer scale (1 to 100 nm). Meanwhile, biotechnology deals with metabolic and other physiological developments of biological subjects including microorganisms. These microbial processes have opened up new opportunities to explore novel applications, for example, the biosynthesis of metal nanomaterials, with the implication that these two technologies (i.e., thus nanobiotechnology) can play a vital role in developing and executing many valuable tools in the study of life. Nanotechnology is very diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale, to investigating whether we can directly control matters on/in the atomic scale level. This idea entails its application to diverse fields of science such as plant biology, organic chemistry, agriculture, the food industry, and more.

Nanobiotechnology offers a wide range of uses in medicine, agriculture, and the environment. Many diseases that do not have cures today may be cured by nanotechnology in the future. Use of nanotechnology in medical therapeutics needs adequate evaluation of its risk and safety factors. Scientists who are against the use of nanotechnology also agree that advancement in nanotechnology should continue because this field promises great benefits, but testing should be carried out to ensure its safety in people. It is possible that nanomedicine in the future will play a crucial role in the treatment of human and plant diseases, and also in the enhancement of normal human physiology and plant systems, respectively. If everything proceeds as expected, nanobiotechnology will, one day, become an inevitable part of our everyday life and will help save many lives.

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Preface

Plants constitute an important trophic level for terrestrial ecosystems and are closely dependent on their environment; their growth is very precisely regulated by a set of ecological factors (light, nutrients, contaminants, and temperature) that can alter the presence, development, and spread of plant species within a territory. The most important factor affecting plant growth and development is the widespread existence of organic and inorganic xenobiotics and contaminants, which is arousing global concern because these agents may influence human and environmental health. Heavy metals are currently the most common inorganic pollutants in the environment and have pronounced effects and consequences not only for plants but also for the ecosystem in which plants form an integral part. Such pollutants have been suggested to accumulate in agricultural crops, thus entering the food chain and posing a significant health risk. Plants growing in the polluted sites exhibit altered metabolism, reduced growth, and decreased biomass production. These pollutants adhere to plant roots and exert physical or chemical toxicity and subsequently cell death in plants. However, plants have developed various defense mechanisms to counteract the toxicity induced by heavy metals.

Only detailed study of the processes and mechanisms would allow researchers and students to understand the interactions, responses, and adaptations of plants to these pollutants; however, there are several unresolved issues and challenges related to heavy metals' interaction and biological impacts. The purpose of this book is therefore to provide important, state-of-the-art findings on environmental phytotoxicity and the mechanisms at the cellular and molecular levels of such interactions. Being interested in this field we usnderstand that knowledge on the cellular and molecular toxicity caused by heavy metals in plants is still elusive, and there is no single book on this particular aspect.

This volume contains several chapters on relevant topics contributed by experts working in the field of environmental phytotoxicity so as to make available a comprehensive treatise designed to provide an in-depth analysis of the topic in question. The book is a collection of numerous chapters with specific text, tables, and illustration explaining the experimental work on phytotoxicity of heavy metals and current trends are reported and some general conclusions are drawn by the contributors as well.

This book serves as a guide for scientists, researchers, and students in the fields of environmental toxicology, phytotoxicology, plant biology, plant physiology, plant biochemistry, and plant molecular biology and those who are interested in toxicity to heavy metals.

We are extremely grateful to all the contributors who warmly welcomed our invitation and agreed to contribute chapters to embellish information on phytotoxicity of heavy metals, thus helping in this endeavor.

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February 03, 2020

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Heavy Metals: Definition, Toxicity, and Uptake in Plants

Cristina Buzea and Ivan Pacheco

Contents

1 Introduction

The industrialization and urbanization during the last century have resulted in increasing accumulation of heavy metals in soils, water, and air, with subsequent uptake of heavy metals by crops. The consumption of increasing amounts of heavy metals from crops poses an important health risk to animals and humans. While several metals are essential for life, some heavy metals and their compounds are having deleterious health effects.

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2 What Are Heavy Metals?

2.1 Heavy Metal Definition

During the years, the inconsistent use of "heavy metal" terminology has led to confusions about the meaning of this term. Therefore, before using it one should comprehensively define it. Before going into more details on the definition of "heavy metals," let us have a look at the definition of "metals," "metalloids," "essential metals," and "micronutrients."

A ***"metal"*** is a material that conducts electricity, is malleable and ductile, has a metallic luster, and forms cations and oxides (Ali and Khan 2018). Having a look at the periodic table of elements, most of the elements are classified as metals. One should also specify the particular conditions for the existence of an element as a metal, such as room temperature and normal pressure. Otherwise, the category of "metals" would include elements that are nonmetal at normal pressure and room temperature but become metallic at higher pressures or at low temperatures (Buzea and Robbie 2005). In addition, the term "metal" is also used by scientists to refer to both the chemical element and its compounds, sometimes without differentiating between the two (Duffus 2002).

A ***"metalloid"*** or a semimetal is an element with properties intermediate between those of typical metals and nonmetals. Metalloids behave chemically like a nonmetal, being electrical insulators at room temperature, and they acquire metallic behavior either after heating, when in small amounts, or when other atoms are intercalated within their structure. Goldsmith reviews most mentioned metalloids as being B, Si, As, Ge, Sb, and Te (Goldsmith 1982). Vernon's list of metalloids includes B, Si, Ge, As, Se, Sb, Te, Po, and At (Vernon 2013). Vernon defined a metalloid as an element with an electronic band structure of a semiconductor or a semimetal, a medium value of first ionization potential (between 750 and 1000 kJ/mol), and a medium electronegativity value (between 1.9 and 2.2) (Vernon 2013).

An ***"essential metal"*** is a metal necessary for a complete life cycle of a living organism (Duffus 2002). When in insufficient amounts, it results in deficiency symptoms. The term refers to both the metal and its compounds.

Another term, mostly used in life sciences, is ***micronutrient***, which is an element with essential functions in plant cells (Appenroth 2010a). Among these are cobalt, copper, iron, manganese, molybdenum, nickel, and zinc. When the concentration of these micronutrients inside a plant exceeds specific thresholds they become toxic.

The earliest usage of the term" heavy metals" seems to belong to a 1936 book of Niels Bjerrum—Inorganic Chemistry third Danish edition (Bjerrum 1936; Foster 1936; Ali and Khan 2018). Bjerrum defined heavy metals as metals with a density higher than 7 g/cm^3 (Ali and Khan 2018). In the following years Bjerrum's definition was changed by modifying the minimum density of a metal that would qualify as a "heavy metal." This limit varied along the years from 3.5 g/cm^3 up to 6 g/cm^3 (Duffus 2002, 2003). To this day there is no consensus to what the minimum density of a "heavy metal" should be.

As time passed, scientists realized that the density of an element does not dictate its reactivity, and perhaps one needs more criteria for defining a "heavy metal." Consequently, another criterion was introduced—the ***atomic weight*** of an element (Duffus 2003). While some authors consider "heavy metals" having atomic weights larger than 23 (starting with magnesium), most authors consider atomic weights exceeding 40 (starting with scandium). If scandium is considered a "heavy metal" under the atomic weight criterion, its density of only 3 g/cm^3 does not qualify it as a "heavy metal" under the density criterion.

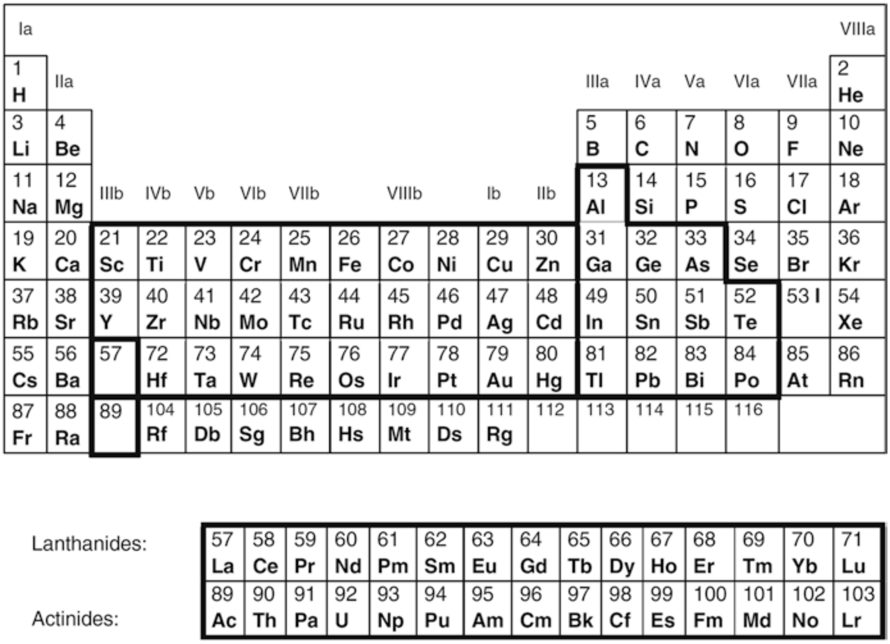
The ***atomic number*** is another criterion for "heavy metal" classification (Duffus 2002). Within this regard, there is more consistency with various authors agreeing upon the rule of atomic numbers higher than 20 (or higher than Ca). However, metals with atomic number higher than 20 include ***essential metals*** that are necessary for the life cycle of an organism, such as Mg and K (Duffus 2002). Abiding by the atomic number criterion, some authors include within the "heavy metals" category the metalloids As and Te, and the nonmetal Se (Ali and Khan 2018; Duffus 2002).

Currently, the term "**heavy metal**" describes metals and metalloids with a high density, the minimum threshold value differing from author to author (Duffus 2002; Ali and Khan 2018). The elements that are usually considered "heavy metals" are shown in Fig. 1, comprising transition metals (middle), rare earth metals (bottom), and lead-group elements (right side) (Appenroth 2010b). Some authors suggest that "heavy metals" should be defined as naturally occurring metals with an atomic number Z larger than 20 and density above 5 g/cm^3 (Ali and Khan 2018). This definition would encompass 51 elements, as depicted in Fig. 2.

2.2 Negative Connotation Associated with "Heavy Metals"

Broadly speaking, scientific literature uses the terminology "heavy metals" as a negative connotation, in association to environmental contamination and pollution, eco-toxicity, and adverse health effects (Duffus 2002; Ali and Khan 2018; Zaidi et al. 2012; Zhou et al. 2016). The term is often used in ecotoxicology, environmental chemistry, medicine, and legislation publications, sometimes without specifying which elements encompass "heavy metals" (Tchounwou et al. 2012; Mustafa and Komatsu 2016; Zwolak et al. 2019). Occasionally, the generic term of "heavy metals" has been used for toxic elements, such as Cd, Hg, and Pb, and other times for elements that are not necessarily metals nor very heavy or dense, such as As and Se (Duffus 2002). Other times, publications will include light elements as heavy metals, such as Cs, Sr, and Ba (Ali and Khan 2018). For example, Ba is an alkaline earth metal with a density of only 3.62 g/cm^3.

One must emphasize that the general assumption that all "heavy metals" are toxic is not supported by scientific evidence. The elements that are under the umbrella of "heavy metals" terminology have different physicochemical, biological, and toxicological properties (Duffus 2002).



Ia																	VIIIa
1 H	IIa											IIIa	IVa	Va	VIa	VIIa	2 He
3 Li	4 Be											5 B	6 C	7 N	8 O	9 F	10 Ne
11 Na	12 Mg	IIIb	IVb	Vb	VIb	VIIb		VIIIb		Ib	IIb	13 Al	14 Si	15 P	16 S	17 Cl	18 Ar
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba	57	72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra	89	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg	112	113	114	115	116		

Lanthanides:	57 La	58 Ce	59 Pr	60 Nd	61 Pm	62 Sm	63 Eu	64 Gd	65 Tb	66 Dy	67 Ho	68 Er	69 Tm	70 Yb	71 Lu
Actinides:	89 Ac	90 Th	91 Pa	92 U	93 Np	94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

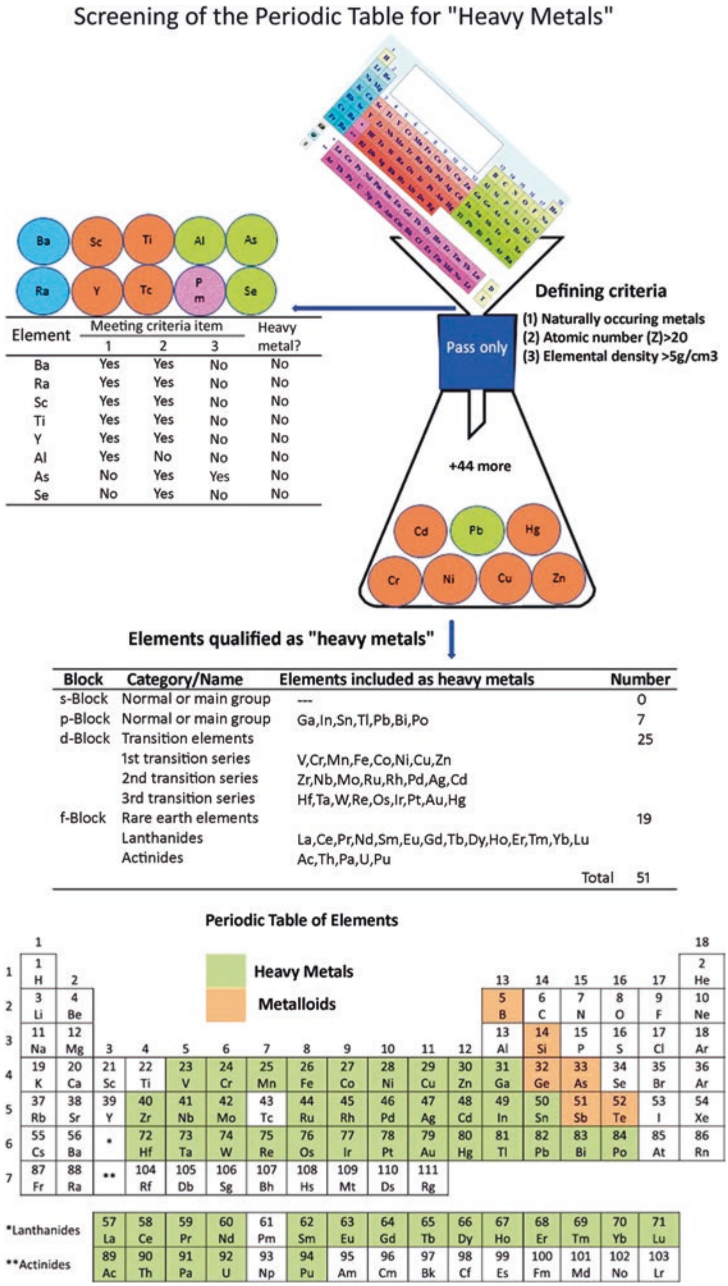
Fig. 1 Schematics showing heavy metals within the periodic table of elements according to Appenroth (reprinted by permission from Springer Nature, Acta Physiologiae Plantarum, "What are heavy metals in Plant Sciences?", vol. 32, pp. 615, Appenroth, K.-J., Copyright (2010) (Appenroth 2010b))

2.3 *Controversy Surrounding the Terminology of "Heavy Metals"*

Duffus suggests that we should give up the term "heavy metals" in favor of a new classification based on the periodic table that reflects toxic effects and can predict them (Duffus 2002).

One counterargument against this idea is that we cannot possibly group the elements in the periodic table based on their toxic effects, not even based on their physicochemical characteristics. Are we considering the elements of the periodic table in bulk form or ionic form, or as microparticles or nanoparticles? Recent toxicological studies reveal the fact that materials believed to be nontoxic in bulk form have high toxicities when in nanoform (Buzea et al. 2007). Even when talking about the same element in ionic form, or as a nanoparticle, its toxicity depends on the chemical corona and its interaction with the biological fluids within an organism. In addition, the same element but in different oxidation states can be either toxic or beneficial for health, like the example of cerium oxides (Pacheco and Buzea 2018).

Moreover, the physicochemical properties of the same element in bulk form can differ essentially from its counterpart in nanoform (Buzea and Pacheco 2017). For



Element	Meeting criteria item 1	2	3	Heavy metal?
Ba	Yes	Yes	No	No
Ra	Yes	Yes	No	No
Sc	Yes	Yes	No	No
Ti	Yes	Yes	No	No
Y	Yes	Yes	No	No
Al	Yes	No	No	No
As	No	Yes	Yes	No
Se	No	Yes	No	No

Block	Category/Name	Elements included as heavy metals	Number
s-Block	Normal or main group	---	0
p-Block	Normal or main group	Ga,In,Sn,Tl,Pb,Bi,Po	7
d-Block	Transition elements		25
	1st transition series	V,Cr,Mn,Fe,Co,Ni,Cu,Zn	
	2nd transition series	Zr,Nb,Mo,Ru,Rh,Pd,Ag,Cd	
	3rd transition series	Hf,Ta,W,Re,Os,Ir,Pt,Au,Hg	
f-Block	Rare earth elements		19
	Lanthanides	La,Ce,Pr,Nd,Sm,Eu,Gd,Tb,Dy,Ho,Er,Tm,Yb,Lu	
	Actinides	Ac,Th,Pa,U,Pu	
		Total	51

🟩 Heavy Metals
🟧 Metalloids

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1 H																	2 He
2	3 Li	4 Be											🟧 5 B	6 C	7 N	8 O	9 F	10 Ne
3	11 Na	12 Mg											13 Al	🟧 14 Si	15 P	16 S	17 Cl	18 Ar
4	19 K	20 Ca	21 Sc	22 Ti	🟩 23 V	🟩 24 Cr	🟩 25 Mn	🟩 26 Fe	🟩 27 Co	🟩 28 Ni	🟩 29 Cu	🟩 30 Zn	🟩 31 Ga	🟧 32 Ge	🟧 33 As	34 Se	35 Br	36 Ar
5	37 Rb	38 Sr	39 Y	🟩 40 Zr	🟩 41 Nb	🟩 42 Mo	43 Tc	🟩 44 Ru	🟩 45 Rh	🟩 46 Pd	🟩 47 Ag	🟩 48 Cd	🟩 49 In	🟩 50 Sn	🟧 51 Sb	🟧 52 Te	53 I	54 Xe
6	55 Cs	56 Ba	*	🟩 72 Hf	🟩 73 Ta	🟩 74 W	🟩 75 Re	🟩 76 Os	🟩 77 Ir	🟩 78 Pt	🟩 79 Au	🟩 80 Hg	🟩 81 Tl	🟩 82 Pb	🟩 83 Bi	🟩 84 Po	85 At	86 Rn
7	87 Fr	88 Ra	**	104 Rf	105 Db	106 Sg	107 Bh	108 Hs	109 Mt	110 Ds	111 Rg							

*Lanthanides	🟩 57 La	🟩 58 Ce	🟩 59 Pr	🟩 60 Nd	61 Pm	🟩 62 Sm	🟩 63 Eu	🟩 64 Gd	🟩 65 Tb	🟩 66 Dy	🟩 67 Ho	🟩 68 Er	🟩 69 Tm	🟩 70 Yb	🟩 71 Lu
**Actinides	🟩 89 Ac	🟩 90 Th	🟩 91 Pa	🟩 92 U	93 Np	🟩 94 Pu	95 Am	96 Cm	97 Bk	98 Cf	99 Es	100 Fm	101 Md	102 No	103 Lr

Fig. 2 Image depicting selection of heavy metals and metalloids from the elements of the periodic table as proposed by Ali and Khan (reprinted by permission from Springer Nature: Toxicological & Environmental Chemistry, “What are heavy metals? Long-standing controversy over the scientific use of the term ‘heavy metals’—proposal of a comprehensive definition”, Ali H. and Khan E., vol. 100, pp. 6–19, Copyright (2018))

example, certain metals nonmagnetic in bulk exhibit magnetic behavior in nanoform, among them being Au, Pt, and Pd (Buzea and Pacheco 2017).

Given the fact that the chemical elements and their compounds under the umbrella term of "heavy metals" have no common toxicity denominator and are selected according to criteria abiding by arbitrary numbers, i.e., the minimum atomic number or elemental density, one should always specify what we call "heavy metals." The meaning of the term "heavy metal" implies that it has a high density; however this physical property is irrelevant in the context of their interaction with plants and organisms (Appenroth 2010a). Moreover, because the term of "heavy metals" includes the metals together with their compounds, one must also underline that as soon as a metal forms a compound, its physical and chemical properties change.

This group of elements termed as heavy metals do not have the same toxicity to organisms, some of them being essential metals necessary for life. There is no correlation between the density of a metal or metal compound and its toxicity to organisms (Appenroth 2010a). The presence in the soil of some metals with a moderate to high atomic number (Cu, Zn, Ni, Pb) can prevent the growth of plants with the exception of a few tolerant species. Hence, the usage of a general term, such as "heavy metals," can be seen as justified for the sake of brevity.

3 Biologically Significant Chemical Properties of Heavy Metals

When speaking about metal toxicity one must take into account their chemical speciation, their biological uptake selectivity, and their biologically significant chemical properties (Duffus 2002). Based on the last electron subshell in the atom to be occupied, metals can be classified as s-block, p-block, d-block, and f-block (Duffus 2002). The s-block metals, such as alkali, form weak complexes acting as electrolytes, while alkaline earth metals are more stable and act as structure promoters and enzyme activators (Duffus 2002). Within the p-block, the higher atomic number metals bind to sulfur, resulting in toxicity. The d-block metals have a wide redox behavior and number of complexes, acting as enzyme catalysts. Within the f-block, comprised of lanthanides and actinides, some metals may act as pollutants.

An important property of a metal ion is its ability to form complexes (Appenroth 2010a). Metals and metalloids are classified in three classes, as class A elements, borderline elements, and class B elements. Hence, the elements that we call "heavy metals" will be divided as:

- Hard acceptors or class A elements: Al^{3+}, Ga^{3+}, Sc^{3+}, Y^{3+}; interact with oxygen-containing ligands.
- Borderline elements: Ga^{3+}, In^{3+}, Sn^{4+}, Pb^{2+}, As^{3+}, Sb^{3+}, Ti^{2+}, V^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}.

- Soft acceptors or class B elements: Tl^+, Tl^{3+}, Pb^{4+}, Bi^{3+}, Pd^{2+}, Pt^{2+}, Cu^+, Ag^+, Au^+, Hg^{2+}. Some of class B ions have high toxicity (Ag^+, Tl^+, Hg^{2+}, Cd^{2+}) and form bonds with sulfur- and nitrogen-containing ligands.

4 Toxicity of Heavy Metals to Humans and Animals

The most commonly environmentally available heavy metals are As, Cd, Cr, Cu, Pb, Ni, and Zn.

Some heavy metals in low concentrations are essential for biochemical and physiological processes within living organisms; however they may become toxic when in higher concentrations (Jaishankar et al. 2014).

One must emphasize that several heavy metals are extremely toxic even at low levels of exposure (Tchounwou et al. 2012). These systemic toxicants are arsenic, cadmium, chromium, lead, and mercury, and can induce multiple-organ damage.

Below we show toxicity effects associated with some of these elements and their compounds, in alphabetical order. Table 1 summarizes the heavy elements and their compounds, their target organs and manifestation, and carcinogenicity in rats and humans (Borm et al. 2004; Kusaka et al. 2001; Guha et al. 2017).

Arsenic: Arsenic, a very abundant element on earth, actually a semimetal but still considered a heavy metal, is toxic and carcinogenic. It affects cell respiration, cell enzymes, and mitosis (Jaishankar et al. 2014).

Lead: High levels of lead are associated to a myriad of health effects, including reproductive toxicity, developmental effects, neurotoxicity, renal dysfunction, lowered immune response, endocrine dysfunction, and hematological effects (Gidlow 2015). Table 2 shows a list of these health effects in males and females.

Mercury: Mercury in the form of a simple element, inorganic salts, and organic compounds has different levels of toxicity. Mercury compounds can be found as water contaminants. Mercury is neurotoxic and is involved in mitochondrial damage and lipid peroxidation. It can also affect kidneys and muscles.

Cadmium: Cadmium is very toxic among heavy metals, causing hepatotoxicity and nephrotoxicity (Jaishankar et al. 2014).

Chromium: Chromium in both its trivalent Cr+3 and hexavalent Cr+6 states is toxic to organisms, including animals, humans, and plants (Jaishankar et al. 2014). It leads to oxidative stress, DNA, and protein damage.

Iron: Iron, the second most abundant metal in earth's crust, is essential for the existence of all organisms. However, iron can damage DNA, mitochondria, and other organelles as a result of free radical production (Jaishankar et al. 2014).

Table 1 Occupational exposure particles and their carcinogenicity according to several sources: the International Agency for Research on Cancer (IARC) *http://monographs.iarc.fr/ENG/Classification/*, Borm P. J. A. et al., Inhaled particles and lung cancer, part B: Paradigms and risk assessment, International Journal of Cancer, vol. 110 (2004) pp. 3–14, Copyright (2004) with permission from John Wiley & Sons, Inc. (Borm et al. 2004); Kusaka Y. et al., Metal-induced lung disease: lessons from Japan's experience, Journal of Occupational Health vol. 43 (2001) pp. 1–23, under a Creative Commons Attribution (CC-BY) License (Kusaka et al. 2001; Guha et al. 2017)

Material and compounds	Target organs or manifestation	Exposure	Carcinogen in rats	Carcinogen in humans
Air pollution	Lung, bladder cancer	Outdoor air pollution	Yes	Yes
Cd	Cancer of lung, kidney, prostate	Metal industry	Yes	Yes
Cr(VI)	Lung, nose, sinuses, cancer	Metal industry	Yes	Yes
Co	Pulmonary fibrosis, lung cancer, DNA damage	Mining, coloring agents, magnetic alloys, industrial and military application		Possibly
Fe	Cancer of the lung Pneumoconiosis	Steel Pigments, diagnosis	Yes	Yes
Pb	Systemic intoxication (blood and central nervous system)	Mining, leaded gasoline, paints, industry		Probably
Mn	Systemic intoxication, neurological diseases	Welding, metal industry		Yes
Ni	Lung and nasal cancer	Mining, milling, smelting, refining	Yes	Yes
Ti	Pneumoconiosis, lung cancer	Pigments, cosmetics, sunscreen agents	Yes	Possibly
V	Asthmatic bronchitis	Mining, refining, alloys, chemical industry	Yes	Possibly

5 Heavy Metal Availability in Soils

Anthropogenic activities have been identified as sources of heavy metal pollution. These include mining, paper mills, cement factory, and metallurgic activities for soil contamination with metals like copper, zinc, lead, and cadmium (Zhang et al. 2009; Cobb et al. 2000). In addition, studies show that vegetables irrigated with wastewater contain a substantial increase of heavy metals, like manganese, iron, copper, and zinc (Arora et al. 2008; Antisari et al. 2015), and chromium, cadmium, nickel, and lead (Ghosh et al. 2012).

Figure 3 shows a schematic comprising environmental availability of heavy metals within soil, followed by the biological uptake of heavy metals, with subsequent accumulation and toxicity within living systems (Kim et al. 2015), (Harmsen 2007). The amount of heavy metals which is environmentally available within the soil comprises the fraction dissolved in the pore water together with the amount already

Table 2 Possible health effects associated with various lead (Pb) levels in blood in humans (table taken from Gidlow D. A, Lead toxicity. Occupational medicine (Oxford, England), 2015, vol. 65, pp. 348–356, by permission of Oxford University Press (Gidlow 2015))

Blood levels (μg)	Males	Females
<5	Nil	Nil
5–10	Possible hypertension and kidney dysfunction	Possible hypertension and kidney dysfunction Possible spontaneous abortion
11–20	Possible hypertension and kidney dysfunction Possible subclinical neurocognitive deficits	Possible hypertension and kidney dysfunction Possible subclinical neurocognitive deficits Reduced birth weight Possible postnatal developmental delay
21–29	Hypertension and kidney dysfunction Possible subclinical neurocognitive deficits	Hypertension and kidney dysfunction Possible subclinical neurocognitive deficits Possible spontaneous abortion Reduced birth weight Possible postnatal developmental delay
30–39	Hypertension and kidney dysfunction Possible neurocognitive deficits	Hypertension and kidney dysfunction Possible neurocognitive deficits Spontaneous abortion Reduced birth weight Possible postnatal developmental delay
40–79	Hypertension and kidney dysfunction Subclinical peripheral neuropathy Neurocognitive deficits Anemia Sperm abnormalities Colic Possible gout	Hypertension and kidney dysfunction Subclinical peripheral neuropathy Neurocognitive deficits Anemia Colic Possible gout Spontaneous abortion Reduced birth weight Possible postnatal developmental delay
80+	Hypertension Nephropathy Peripheral neuropathy Neurocognitive deficits Anemia Sperm abnormalities Colic Gout Encephalopathy	Hypertension Nephropathy Peripheral neuropathy Neurocognitive deficits Anemia Colic Gout Encephalopathy Spontaneous abortion Reduced birth weight Possible postnatal developmental delay

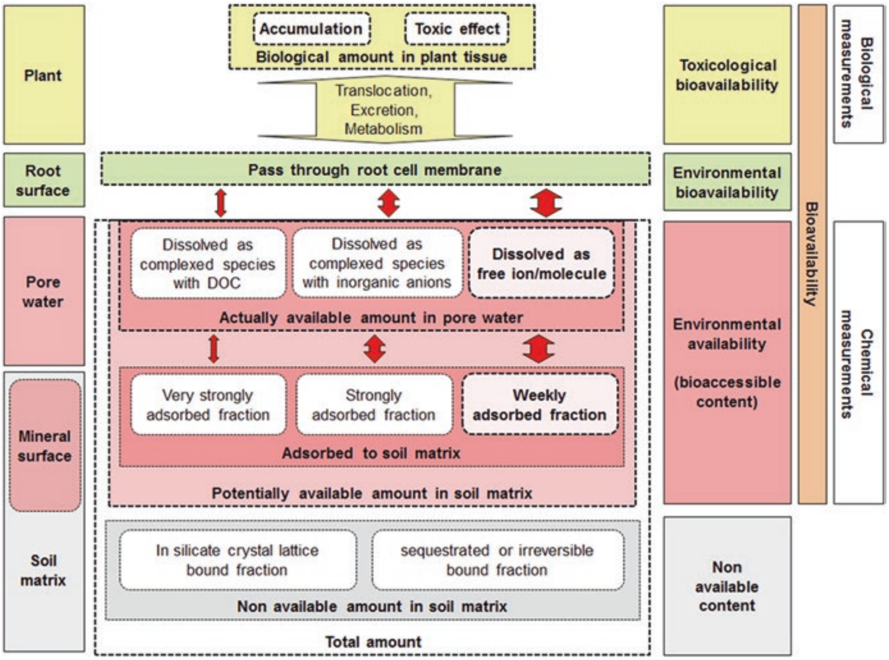


Fig. 3 Schematics depicting the concept of heavy metal bioavailability in plants from soils. The thickness of the arrows correlates with the importance in affecting bioavailability (image reprinted by permission from Springer, Environmental Geochemistry and Health, Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review, Kim et al. (2015))

adsorbed within the soil particles (Kim et al. 2015). The uptake amount of heavy metals from the soil is not a fixed fraction, but should be regarded as a function of the exposure time. The heavy metals potentially available for uptake have various desorption kinetics, depending on their chemistry as well as the soil properties, such as pH and texture.

Table 3 shows the most frequent species of metal ions dissolved within the pore water of agricultural and forest soil. The complexes of heavy metals with inorganic anions like Cl−, OH−, and HCO3− in soils with intermediate to alkaline pH are generally believed to foster bioavailability in plants. The fraction of environmentally bioavailable heavy metals is the fraction that is dissolved in the pore water and can suffer uptake by plant roots. This depends on metal chemistry and plant physiology and can differ among plant species (Kim et al. 2015). High metal ion concentration in pore water can lead to predominantly passive uptake, while lower metal ion concentrations suffer a metabolic active uptake (Kim et al. 2015). The type of metal also dictates the type of uptake: Cd, Cr(III), Ni, and Pb suffer mainly a passive uptake, while the uptake of essential plant nutrients, such as Cu and Zn, may be via an active or both passive and active uptake (Kim et al. 2015).

Table 3 Regular concentrations of heavy metal ions dissolved in the pore water for low or non-contaminated acidic forest soil and agricultural soils

	Pore water concentrations		Species	
	Acidic forest soil (µg/L)	Agricultural soil (µg/L)	Very strong acidic to moderately acidic soils	Slightly acidic to alkaline soils
Cd	1–25	<0.1–3	Cd^{2+}, $CdSO_4^0$, $CdCl^+$	Cd^{2+}, $CdSO_4^0$, $CdCl^+$
Cr	2–20	<1–15	Cr^{3+}, $CrSO_4^+$, Cr-DOC	Cr-DOC, $CrCO_3^+$, $Cr(CO_3)_2^-$, $Cr(CO_3)_3^{3-}$
Ni	5–30	1–30	Ni^{2+}, $NiSO_4^0$, Ni-DOC	$NiCO_3^0$, $NiHCO_3^+$, $NiB(OH)_4^+$
Cu	1–50	3–60	Cu-DOC, Cu^{2+}, $CuSO_4^0$	Cu-DOC, $CuCO_3^0$, $CuB(OH)_4^+$
Pb	2–100	<1–50	Pb^{2+}, Pb-DOC, $PbSO_4^0$	$PbCO_3^0$, $PbHCO_3^+$, $Pb(CO_3)_2^{2-}$
Zn	80–2000	10–400	Zn^{2+}, $ZnSO_4^0$	$ZnHCO_3^+$, Zn^{2+}, $ZnSO_4^0$, $ZnCO_3^0$

DOC dissolved organic carbon

Reprinted by permission from Springer, Environmental Geochemistry and Health, Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review, Kim et al. (2015)

Table 4 Transfer factors of heavy metals from soil to plants in contaminated soils, concentration range in mature leaf tissue, safety limits in foodstuff. *DW* dry weight, *FW* fresh weight (reprinted by permission from Springer, *Environmental Geochemistry and Health,* Bioavailability of heavy metals in soils: definitions and practical implementation—a critical review, Kim et al. (2015))

		Concentration range (mg/kg DW)			Safety limit (mg/kg FW)		
	Transfer factor	Deficient	Normal	Toxic	Leaf vegetables	Stem/root vegetables	Rice
Cd	1–10	–	0.05–0.2	>5–10	0.2	0.1	0.2
Zn	1–10	<10–25	25–150	>150–400	–	–	–
Ni	0.1–1	–	0.1–5	>20–30	–	–	–
Cu	0.1–1	<2–5	5–20	>20–100	–		–
Cr	0.01–0.1	–	0.1–0.5	>1–2	–	–	–
Pb	0.01–0.1	–	1–5	>10–20	0.3	0.1	0.2

The soil physicochemical properties are a decisive factor in the heavy metal concentration in soils available for plant uptake. Metal solubility in soil is dictated by the pH value and the percentage of clay in the soil (Golia et al. 2008).

6 Transfer Factor of Heavy Metals from Soil to Plants

The transfer factor of heavy metals is a measure that predicts the amount of heavy metals accumulated within plants from soils. The transfer factor depends on the type of plant, the type of soil, and the type of heavy metal. For example, leafy vegetables have a higher accumulation of metals compared to root vegetables which have a moderate uptake, while legumes have the lowest accumulation (Alexander et al. 2006). The transfer factor of metals increases from Pb and Cr to Ni and Cu, with the highest being for Cd and Zn (Kim et al. 2015). Table 4 shows usual metal transfer factors from soil to plants, concentration ranges in plant leaves, safety limits, and trigger values for adverse effects for arable soils for a series of metals: Cd, Cu, Cr, Ni, Pb, and Zn (Kim et al. 2015).

7 Genotypic Variations in the Accumulation of Heavy Metals

The uptake concentration of heavy metals in plants is a function of plant species, the variety type within the same species, and the location in plant tissue (Alexander et al. 2006; Zwolak et al. 2019; Pajević et al. 2018; Nikolić et al. 2014; Zhou et al. 2016).

An experiment involving various cultivars of several vegetables grown in control soil and in soil with higher amounts of Cd, Cu, Pb, and Zn shows various uptake of heavy metals for different plant species, and even within the same species for different cultivars (Alexander et al. 2006). The results are summarized in Table 5 (Alexander et al. 2006). Various cultivars of the same vegetable show differences in the amount and type of heavy metals accumulated. For example, Amsterdam carrots accumulate higher concentrations of Cd, Cu, and Zn than Ingot carrots, while the opposite happens for Pb. When comparing one vegetable to another, the legumes have the least accumulation of metals, root vegetables have a moderate accumulation while leafy vegetables have uptake of the highest concentrations of heavy metals (Alexander et al. 2006).

Adults and children might have an increased health risk due to exposure to heavy metals from consumption of vegetables grown on contaminated farmland. Vegetables grown on farmland contaminated with Pb, Cd, Cu, Zn, and As show different uptake of heavy metals (Zhou et al. 2016). The concentration of heavy metals was found to be the highest for leafy vegetables, and decreased for stalk/root/solanaceous vegetables, with the lowest concentrations for legumes/melon vegetables (Zhou et al. 2016). Table 6 shows the concentrations of heavy metals in vegetable edible parts grown in Shizhuyuan area, China, the National Standard value which is the tolerance limit of contaminants in foods in China according to the China National Standards (GB 2762-2012) (Zhou et al. 2016). Data includes mean ± standard error of three replicates.

Table 5 Mean metal concentration in vegetable grown in control and heavy metal-treated soil in the UK. The ES-European Standards values are taken from reference Zwolak et al. (2019)). Table adapted from Environmental Pollution, Vol. 144, Alexander P. D. et al., "Genotypic variations in the accumulation of Cd, Cu, Pb and Zn exhibited by six commonly grown vegetables", pp. 736–745, Copyright (2006), with permission from Elsevier (Alexander et al. 2006)

		Cadmium		Copper		Lead		Zinc	
Vegetable species	Vegetable cultivar	Treated	Control	Treated	Control	Treated	Control	Treated	Control
Carrot	Amsterdam	2.521	0.068	5.77	2.78	6.31	0.34	52.59	19.68
	Nantes	1.812	0.054	4.43	2.42	5.01	0.17	42.54	19.02
	Ingot	2.298	0.074	4.43	2.81	7.23	0.42	33.38	18.43
	European Standards	0.1		–		0.1		–	
Spinach	Bloomsdale	4.24	0.36	9.01	6.29	1.74	0.62	334.9	246.7
	Grodane	6.94	0.45	10.08	6.44	1.78	0.69	380.2	209
	Mediana	6.48	0.33	8.97	7.31	2	0.51	343.2	256
	European Standards	0.3		–		0.3		–	
Pea	Douce P	0.4282	0.1054	5.163	4.117	1.399	0.412	47.49	32.36
	Feltham	0.2615	0.1055	5.727	4.189	0.547	0.322	47.8	36.83
	Fortune	0.3355	0.1441	6.617	4.572	0.731	0.692	54.27	36.68
	European Standards	0.05		–		0.2		–	
Onion	Buffalo	4.0229	0.1928	2.374	2.307	6.486	1.689	50.45	23.75
	Express	3.5566	0.2237	3.078	2.755	8.745	1.216	60.94	32.28
	Keepwell	3.3498	0.1107	2.878	2.342	6.519	1.125	66.97	16.25
	European Standards	0.2		–		0.3		–	
Lettuce	Corsair	9.033	0.144	12.55	5.67	11.73	0.06	160.69	51.24
	Little gem	8.173	0.233	9.97	7.07	19.68	2.9	172.17	70.59
	Lobjoits	9.083	0.084	8.1	5.35	12.95	1.02	163.88	58.15
	European Standards	0.3		–		0.3		–	

Table 6 Concentrations of heavy metals in vegetables grown in China (reproduced from Zhou H. et al. 2016. International Journal of Environmental Research and Public Health, 13, 289 (CC-BY 4.0) (Zhou et al. 2016))

	Vegetable species	Pb (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	As (mg/kg)
Root (*n* = 3)	White radish	0.270 ± 0.057	0.011 ± 0.003	0.167 ± 0.073	4.690 ± 1.367	0.099 ± 0.012
	Carrot	0.233 ± 0.001	0.023 ± 0.005	0.227 ± 0.011	1.591 ± 0.092	0.188 ± 0.030
	Sweet potato	0.613 ± 0.162	0.135 ± 0.007	0.015 ± 0.005	4.674 ± 0.790	0.448 ± 0.013
	China National Standards	0.1	0.1	/	/	0.5
Stack (*n* = 2)	White cai tai	0.785 ± 0.239	0.239 ± 0.090	0.456 ± 0.033	24.23 ± 5.541	0.225 ± 0.043
	Red cai tai	0.939 ± 0.327	0.176 ± 0.038	0.478 ± 0.089	20.95 ± 2.993	0.396 ± 0.076
	China National Standards	0.3	0.1	/	/	0.5
Solanaceous (*n* = 3)	Eggplant	0.429 ± 0.001	0.289 ± 0.027	0.937 ± 0.199	2.786 ± 0.588	0.072 ± 0.004
	Red pepper	0.056 ± 0.009	0.047 ± 0.035	0.589 ± 0.124	2.241 ± 0.169	0.016 ± 0.005
	Tomato	0.078 ± 0.023	0.028 ± 0.005	0.468 ± 0.036	1.419 ± 0.185	0.014 ± 0.003
	China National Standards	0.1	0.05	/	/	0.5
Melon (*n* = 4)	Cucumber	0.004 ± 0.001	0.004 ± 0.001	0.284 ± 0.047	1.206 ± 0.107	0.039 ± 0.014
	Pumpkin	0.121 ± 0.031	0.005 ± 0.001	0.647 ± 0.104	2.883 ± 0.749	0.073 ± 0.015
	China National Standards	0.1	0.05	/	/	0.5
Leafy (*n* = 2)	Cabbage	0.671 ± 0.277	0.036 ± 0.022	0.314 ± 0.043	9.926 ± 2.226	0.211 ± 0.014
	Chinese cabbage	0.749 ± 0.129	0.419 ± 0.027	0.155 ± 0.024	14.51 ± 2.811	0.073 ± 0.020
	Spinach	0.971 ± 0.194	0.513 ± 0.055	0.966 ± 0.288	20.81 ± 4.543	0.310 ± 0.064
	Caraway	1.855 ± 0.208	1.031 ± 0.064	0.987 ± 0.037	52.69 ± 6.200	0.739 ± 0.075
	Lettuce	1.162 ± 0.540	0.460 ± 0.207	0.775 ± 0.170	11.79 ± 1.688	0.660 ± 0.086
	China National Standards					
Legume (*n* = 2)	Asparagus bean	0.070 ± 0.014	0.013 ± 0.011	1.999 ± 1.247	6.682 ± 1.550	0.047 ± 0.014
	Kidney bean	0.033 ± 0.036	0.010 ± 0.017	1.310 ± 0.085	5.669 ± 0.236	0.050 ± 0.010
	China National Standards					

8 Relative Toxicity of Heavy Metals in Plants

It is impossible to determine a general scale of heavy metal toxicity to living organisms. Quantitative toxicity measurements of various metals did not show any correlations of their position in the toxicity scale with their physical or chemical properties (Appenroth 2010a). One can only give examples of such measurements in specific plants. For example, quantitative measurements of growth inhibition for the plant *Lemna minor*, including multiplication rate, fresh and dry weight, chlorophyll a and b, and total carotenoid content, showed the following sequence of toxicity (Appenroth 2010a):

$$Ag^{+} > Cd^{2+} > Hg^{2+} > Tl^{+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{6+} > Cr^{6+} > As^{3+} > As^{5+}$$

Accumulation of heavy metals in plant tissue is associated with decreased root length and plant biomass, negatively affecting seed germination and chlorophyll biosynthesis (Ahmed et al. 2019; Di Salvatore et al. 2008). At a cellular level heavy metals can detrimentally influence photosynthesis and respiration, and other physiological factors, often as a result of an increased production of reactive oxygen species (Ahmed et al. 2019; Shahid et al. 2014; Dimkpa et al. 2012; Pinho and Ladeiro 2012).

Some of the heavy metal ions are required for an optimal growth and development in plants. These include copper, iron, manganese, molybdenum, and zinc (Anjum et al. 2015). However, an inappropriate amount of these metals can lead to phytotoxicity.

9 Conclusions

Heavy metals have a negative connotation, being associated to environmental contamination and pollution, eco-toxicity, and adverse health effects. While some of the metals termed “heavy metals” are essential for living organisms, others are extremely toxic to humans and animals. The accumulation of heavy metals in plants occurs via uptake from soil and wastewater. The highest uptake of heavy metals occurs in leafy vegetables, followed by a moderate level in root vegetables, and the lowest accumulation in legumes. The consumption of plants with high levels of heavy metals can pose deleterious health effects to humans and animals.

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The Hows and Whys of Heavy Metal-Mediated Phytotoxicity: An Insight

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Contents

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1 Introduction

During the last decade, cellular, molecular, and physiological basis of interaction between crop plants and various environmental stress factors has drawn considerable attention as plants due to their immobile nature confront these stresses throughout their life cycle, thus serving as the main route for the entry of these factors in the food chain, an alarming situation for human health. Heavy metals are one of these factors that can negatively affect plant growth, development, and overall crop productivity by hampering the normal physiological and metabolic processes of plants (Dal Corso et al. 2008; Hossain et al. 2009, 2010; Rascio and Navari-Izzo 2011; Villiers et al. 2011). Heavy metals constitute a heterogeneous group of naturally occurring elements with atomic number 20 or more and with relatively high elemental density (approximately 5 g/cm^3) (Ali and Khan 2017). But in recent days, scientists have been using the term "heavy metals" to refer to those metal and metalloids including copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), nickel (Ni), cobalt (Co), cadmium (Cd), and arsenic (As) which are toxic and exert deleterious effects on plant health (Hossain et al. 2012). Few heavy metals and transition metals, such as Zn, Cu, Mo, Mn, and Co, act as essential micronutrients and at certain concentrations are crucial for a wide range of physiological and developmental pathways because they are critical for the functional activities of certain enzymes involved in these pathways (Zenk 1996; Salla et al. 2011; Shahid et al. 2015). These abovementioned heavy metals and transition metals when present in higher than supraoptimal level and other nonessential heavy metals such as cadmium (Cd), lead (Pb), and mercury (Hg) are highly detrimental to all organisms including plants as they hamper the membrane function and enzyme activities (Xiong et al. 2014; Ding et al. 2017; Shi et al. 2019). Although heavy metals are natural components of soil and present naturally in the environment, in recent few years several anthropogenic activities including mining, modern agricultural practices, and industrialization have caused a sharp increase in heavy metal concentration resulting in soil contamination (Rascio and Navari-Izzo 2011; Hossain et al. 2012). Modern agriculture process itself can serve as a prime reason for cadmium and copper contamination in soil. Several copper-containing compounds are widely used as pesticides and phosphate fertilizers that have been used in the fields for a long time, and serve as the source of Cu and Cd. Irrational use of these pesticides and fertilizers is responsible for gradual increase of soil contamination (Kupper and Andresen 2016).

Toxicity of the heavy metals is dependent on their redox activity. Metal toxicity hampers different physiological activities in crop plants including interference in enzyme activities, denaturation of some important structural and functional proteins, expulsion/replacement of important metal ions from different biomolecules like chlorophyll, and disruption of structural integrity of plasma membrane. All these can cause morphological abnormalities, metabolic disorders, and inhibition of photosynthesis and respiration, ultimately leading to a significant reduction in crop productivity and yield. In addition, heavy metals also hamper the redox homeostasis of the cell by accelerating the production of several reactive oxygen species (ROS) including superoxide anion radical (O^{2-}), H_2O_2, and hydroxyl radical (OH–) (Sharma and Dubey 2007). This elevated level of ROS induces oxidative stress in cells which will disrupt the structure of biological membrane and macromolecules and induce lipid peroxidation and genotoxicity. In response to heavy metal toxicity plants have also developed a network of efficient mechanisms that regulate the uptake, sequestration, mobilization, relocation, and detoxification of harmful heavy metals, thus maintaining their optimum concentration in the cytosol to allow all the metabolic activities.

This chapter summarizes the effects of heavy metal stress on various metabolic and physiological processes in plants which limit their growth and development. Special focus has been given on the current development in the context of understanding heavy metal-mediated phytotoxicity mechanisms with detail information on heavy metal uptake in root cells by various transporters, components associated with translocation, and sequestration of the heavy metal ions.

2 Different Heavy Metals and Metalloids and Their Effect on Plants

2.1 Cadmium (Cd)

Cadmium is a silvery bluish colored group XII element with an atomic weight of 112.411. Due to its hydrophilic nature, Cd is considered as one of the most phytotoxic heavy metals. At very low concentration Cd can inhibit some key enzymes involved in Calvin cycle, CO_2 assimilation, and carbohydrate and nitrogen metabolism (Gill and Tuteja 2011) which ultimately results in stunted growth, leaf epinasty, and disruption of chloroplast structure (Sharma and Dubey 2006). Cd has also been involved in the inhibition of seed germination by the inhibition of a-amylase and invertase enzymes and by the accumulation of lipid peroxidation products (Ahsan et al. 2007). Cd concentration beyond normal level negatively affects the absorption and transport of key macronutrients which results in metabolic disorders (Xu and Shi 2000). The inhibition of DNase and RNase activity along with alteration of nucleolar structure was also observed in Cd-mediated phytotoxicity (Duan and Wang 1992).

2.2 Arsenic (As)

Naturally arsenic is a metalloid compound belonging to the group XV of pnictogens in periodic table with an atomic weight of 74.92. The ground water As contamination not only affects crop productivity but also contaminates the entire food chain (Verma et al. 2016). Recently the interruption of physiological and molecular processes by arsenic is investigated in several plants like rice, lettuce, spinach, and carrot (Kumar et al. 2015). The more toxic form of As, the trivalent arsenite (AsIII), induces DNA cleavage, thereby inducing intrachromosomal homologous recombination (Helleday et al. 2000). Arsenic is also responsible for the inhibition of some key respiratory enzymes via binding to neighboring thiols of pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase (Verma et al. 2016).

2.3 Lead (Pb)

Lead is naturally soft in nature with light grey in color. It belongs to group XIV b in periodic table with an atomic mass of 207.2. Lead is one of the most toxic heavy metals which exist in earth crust in various forms. Lead (Pb) is toxic to both plants and animals due to its nonbiodegradable nature. It inhibits seed germination and viability via alteration of transpiration and genomic DNA profile (Sethy and Ghosh 2013). It also retards the seedling development, chlorophyll biosynthesis, and cell division (Kumar et al. 2017).

2.4 Mercury (Pb)

Naturally mercury is a silvery group XII d block element with an atomic mass of 200.59. The persistent nature and bioaccumulation property of mercury create hazard for the food chain of ecosystem. Mercury has negative effect on photosynthesis and interferes with chloroplast and mitochondrial electron transport chain. The phytotoxic effect of mercury also impairs water uptake via root system by inhibiting aquaporins (water channels in cell membrane) (Zhou et al. 2009). The generation of free radicals under mercury-mediated stress causes cellular injuries and several physiological abnormalities in plants (Zhou et al. 2007).

2.5 Chromium (Cr)

Chromium is a group IV element with standard atomic weight of 51.99 and silvery metallic in appearance. Growth and development of plants are highly affected by trivalent Cr^{3+} and hexavalent Cr^{6+} elements. In general chromium is not directly absorbed by plants; rather they form conjugate with sulfate and iron. The toxic effect of chromium is expressed with the change of soil pH which greatly perturbs the nutrient absorption by roots. In *Helianthus annuus* and *Amaranthus viridis* chromium toxicity results in decrease in root length along with extension of cell cycle (Fozia et al. 2008; Zou et al. 2006). Chromium has the capability to create metabolic disorders associated with seed germination. In previous study, it was observed that the treatment of cowpea (*Vigna sinensis*) seeds with Cr^{6+} markedly affects the amylase activity and the reduction in total sugar content resulting in reduced seed germination (Nath et al. 2008). By inhibiting essential nutrient (Fe, Mn, Ca, Mg, K, P) uptake by root system chromium toxicity leads to the decreased shoot growth and development of leaves (Nematshahi et al. 2012). At high concentration Cr^{6+} (16–32 mg/kg soil) results in degeneration of root tip cells along with drying of leaf margin.

2.6 Manganese (Mn)

The essential micronutrient, transition metal manganese, with an atomic weight of 54.93 plays an important role as a cofactor of several enzymes like isocitrate dehydrogenase, nitrate reductase, and superoxide dismutase (Todorovic et al. 2009; Millaleo et al. 2010), involved in biomolecule (lipid, fatty acid, carotenoid) biosynthesis and carbohydrate and nitrogen metabolism pathways. However in acidic soil pH, Mn^{2+} becomes phytotoxic. The toxicity of manganese is accelerated by different light levels and by different plant species. Manganese toxicity results in chloroplast deterioration in *Pisum sativum* (Rezai and Farboodnia 2008). The phytotoxic effects are pronounced in old and aged leaves where chlorosis associated with brown spots and leaf vein darkening is observed (Maksimovic et al. 2012). In rice and sunflower manganese was responsible for reduced shoot and root growth (Hajiboland and Hasani 2007).

2.7 Nickel (Ni)

The group X element nickel at higher concentration becomes toxic and inhibits seed germination of several crop plants by affecting amylase, protease, and ribonuclease enzymes (Ahmad and Ashraf 2011). Ni toxicity results in decrease in chlorophyll content and thereby negatively affects photosynthetic apparatus (Sreekanth et al. 2013). An increase in MDA content with the concomitant increase in lipid peroxidation was observed in *Zea mays* (Baccouch et al. 2001). Besides these Ni hampers the mitotic cell division by agglomeration reaction in root apex cell plants (L'Huillier et al. 1996).

2.8 Copper (Cu)

The reddish pink-colored copper induces phytotoxic effect via generation of reactive oxygen species (ROS) and reduction of catalase activity (Pena et al. 2011). The acidic pH of soil leads to more accumulation of copper in the roots of cucumber (*Cucumis sativus*) (Alaoui-Sosse et al. 2004). Copper inhibits seed germination by the inhibition of a-amylase, enolase, and invertase isoenzymes and thereby completely disrupts seed metabolism and mobilization of reserve food (Sethy and Ghosh 2013).

2.9 Zinc (Zn)

Zinc is a group XII silvery grey post-transition metal. It was observed in previous studies that zinc (Zn^{2+}) is responsible for decrease in photosynthetic pigments in sorghum and bean (Mirshekali et al. 2012; Vassilev et al. 2011). Excess zinc causes cytotoxic and genotoxic effect on plants. A high level of zinc leads to sticky metaphase and unusual segregation of chromosome with ana-telo abnormality in *Vigna subterranean* and *Hordeum vulgare* seedlings (Oladele et al. 2013; Truta et al. 2013). The toxic effect of zinc is associated with changes in crystals in leaf morphology with the formation of calcium oxalate in poplar and tomato plants (Todeschini et al. 2011; Vijayarengan and Mahalakshmi 2013).

2.10 Aluminum (Al)

Aluminum is a group XIII metalloid compound. There is no significant biological role of aluminum in plant growth. But at acidic pH (~5–5.5) Al^{3+} becomes hazardous to plants (Emamverdian et al. 2015). Generally roots are largely affected by aluminum after absorption and accumulation in root cells along with the thickening of lateral roots. After interacting or binding with cell wall and DNA aluminum imposes structural rigidity, thereby resulting in growth retardation and cell cycle arrest, respectively (Foy et al. 1992). Effects on the aerial parts are largely expressed by the formation of necrotic spots, curling of young leaves, decrease in stomatal aperture, and death of petioles (Bian et al. 2013; Wang et al. 2006). In maize and sorghum transportation of essential mineral elements has been interrupted by aluminum toxicity (Bhalerao and Prabhu 2013). Alteration of chromatin structure along with changes in molecular structure was also observed under aluminum-mediated phytotoxicity.

3 Uptake, Transport, and Relocation of Heavy Metals by Plants

3.1 Uptake of Heavy Metals

Different heavy metals present as ions in the soil solution are accessible to plant roots, and primarily approach and bind to the cell wall of the root cells. These heavy metals were then taken up across the plasma membrane by interacting with the

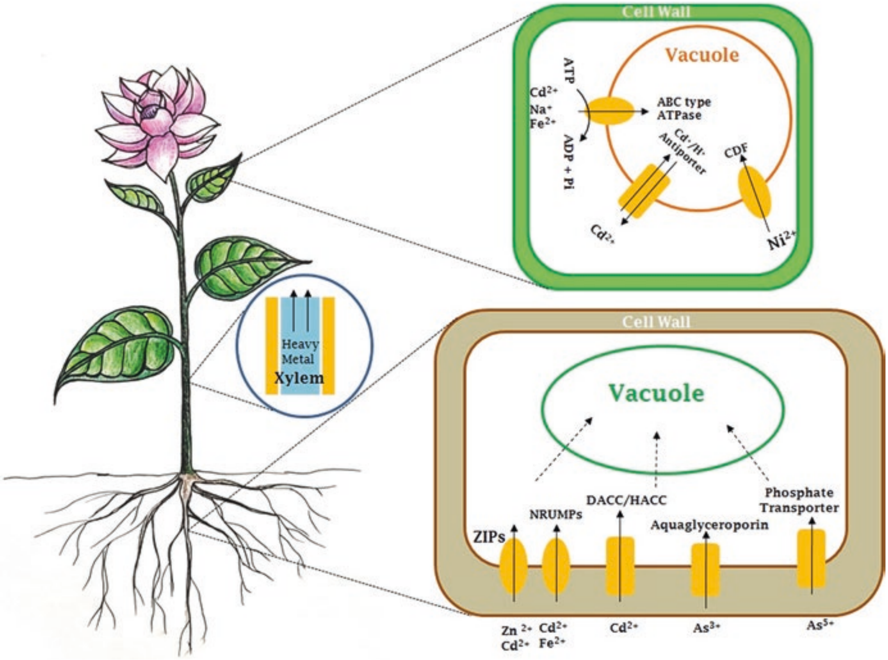

Fig. 1 Schematic representation of uptake and transport of heavy metals in plants through various metal transporters

high-affinity binding sites and carrier system localized in the plasma membrane. Mechanism of uptake of Cd, Pb, and Ni by plants is somewhat different from each other. Uptake of Cd and Ni by root cells takes place by both passive diffusion and active transport (Fig. 1) (Costa and Morel 1993; Seregin and Kozhevnikova 2006). On the other hand, Pb uptake mainly takes place through passive diffusion and is mainly transported in the apoplastic region, i.e., through the intercellular spaces (Tung and Temple 1996). It is interesting to note that along with root, leaves may also involve in heavy metal absorption. Metal ion uptake generally takes place through different secondary transporters like channel proteins and H^{+}-coupled carrier proteins and this is mainly facilitated by the membrane potential of plasma membrane (Hirsch et al. 1998). Cytoplasmic efflux of transition metal ions such as Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, or Mn^{2+} in the root cells takes place via the cation diffusion facilitator (CDF) transporters (earlier named as MTP or metal tolerance protein (Yang and Chu 2011)).

Cd takes entry into the root cells via different calcium channels, such as depolarization-activated calcium channels (DACC) and hyperpolarization-activated calcium channel (HACC) (White 2005; Verbruggen et al. 2009). Transport through both these channels is eased by the negative membrane potential of the inner side of plasma membrane. Cd^{2+} metal ions can also take entry into the root cells by the transporters associated with the transport of essential cations like Fe^{2+}. Besides these, natural resistance-associated macrophage proteins (NRAMP) and ZNT proteins (homolog of zinc-iron permease [ZIP] gene) are also associated with the uptake and transport of Cd^{2+} ions along with mineral nutrients Fe and Zn^{2+}, respectively (Thomine et al. 2000; Pence et al. 2000). Another path for Cd uptake is reported in tobacco in which membrane-bound TaLCT1 transporter regulates both Ca^{2+} and Cd absorption. Pb uptake by plant roots takes place through passive diffusion, although some proteins like NtCBP4 in tobacco may assist this transport process across the plasma membrane (Arazi et al. 2000). It is also reported that Pb and Ni absorption is dependent on the pH of the soil solution and Ni uptake increases with the increase in soil pH up to 8.5. It is interesting to note that unlike Cd, Pb and Ni uptake is inhibited by Ca^{2+} ion (Kim et al. 2002). Arsenic uptake by plant roots depends on its form. Arsenate (AsV) uptake by plant roots takes place via phosphate transporters and arsenite (AsIII) is taken up with the help of aquaglyceroporins (Meharg and Jardine 2003).

3.2 Transport from Root to Shoot

After entering into the root, heavy metals can be either exported to the shoot system or accumulated in the root cells. Heavy metal cations like Cd^{2+}, Pb^{2+}, and Ni^{2+} are further translocated along the transpiration stream to the aerial parts including stem, leaves, flowers, fruits, and seeds (Peralta-Videa et al. 2002; Clemens and Ma 2016). For loading into the xylem of vascular cylinder, the heavy metal ions move centripetally (i.e., from root surface to vascular cylinder) following apoplastic or symplastic pathway (Yin et al. 2015). After reaching the aerial parts, the metal ions in the xylem conduits may further be translocated radially to the phloem cell in both symplastic and apoplastic routes. This translocation of metal ions from underground part to the aerial part through xylem cylinder is tightly regulated by thiol-containing compound phytochelatins. Several studies have demonstrated that HMs can be accumulated along their transport pathways in plants (Clemens and Ma 2016; He et al. 2015; Wang et al. 2015). Organic acids like malic and citric acid modulate Ni^{2+} chelation in xylem cells by providing protons for this process. Apart from phytochelatins and organic acids, several amino acids and peptides including histidine, nicotinamine, and metallothioneins have the capability to act as ligands in the cytoplasm or subcellular compartments for efficient transport, translocation, and accumulation of heavy metal ions within plants. The overall transport and relocation of the metal ions from root to shoot through vascular cylinder require transporters such as P_{1B}-type ATPases, MATESs, and OPTs, which are mainly localized in the parenchyma cells of xylem (Dalcorso et al. 2013).

4 Intracellular Sequestration Within Subcellular Organelle

In plants, cytosolic metal concentration has to be maintained in a balanced manner to cope up with its toxicity. One such strategy is sequestration or compartmentation of heavy metal ions within various cellular components including cell wall, vacuole, and Golgi apparatus. During entry in the plant cell, heavy metal ions first face the barrier of cell wall, where they can bind to the functional groups of different cell wall components, especially cellulose, pectin, and lignin (Chen et al. 2013; Parrotta et al. 2015). Enhancement in lignin biosynthesis has been reported in some plants following exposure to heavy metals suggesting the involvement of lignin in metal sequestration in the cell wall (Cheng et al. 2014; Elobeid et al. 2012). Within the cell, heavy metal ions can be transported to subcellular compartments including vacuole and Golgi apparatus; both of these can serve as reservoir for heavy metal sequestration. In Zn/Cd hyperaccumulator plant species *S. alfredii*, Cd is mainly accumulated in vacuoles of parenchyma cells of leaf mesophyll, stem pith, and cortex (Tian et al. 2017). In barley leaves, elevated accumulation of Zn in the vacuole has been reported when the plants were exposed to increasing concentration of the metal (Brune et al. 1994).

Various channels and pumps present in tonoplast are involved in the uptake of metal ions within the vacuole. Some well-characterized transporter proteins associated with vacuolar transport of heavy metal ions are ATP-binding cassette (ABC) transporters, Cd^+/H^+ antiporter, P-type metal ATPases, natural resistance-associated macrophage protein (NRAMP) family, and cation diffusion facilitator (CDF) family of proteins (Fig. 1) (Chiang et al. 2006; Dubey 2011; Kramer et al. 2007). Golgi apparatus, an important part of the intracellular membrane system, plays a crucial role in the sequestration of Mn^{2+} via vesicular trafficking and exocytosis and thereby reduces the cytosolic Mn^{2+} level as well as Mn toxicity (Erbasol et al. 2013; Peiter et al. 2007).

Vacuolar sequestration of heavy metal ions in the vacuole and Golgi apparatus removes these toxic cations from the cytosol and other cellular organelles including chloroplast and mitochondria, where sensitive metabolic reactions take place, thereby alleviating the toxic effect of heavy metal ions on the enzymes of these reactions.

4.1 Phytochelatins

Phytochelatins (PCs) are thiol group containing metal-binding low-molecular-weight polypeptides, mainly found in photosynthetic organism, ranging from lower group of plants (algae, fungi, gymnosperms) to higher plants (monocots and dicots) (Cobbett 2000). Phytochelatins act as high-affinity ligands forming stable complex with heavy metals and play a crucial role in root-to-shoot translocation and distribution of various heavy metals including Cd and Pb. PCs are a family of Cys-rich polypeptides derived from glutathione (GSH) and carry the general structure (γ-Glu-Cys)n-Gly, in which γ-Ala, Ser, Gln, or Glu and n = 2–11 (Zenk 1996; Cobbett 2000; Yang et al. 2005). Biosynthesis of PCs is carried out from GSH by the constitutively expressed γ-glutamylcysteinyl dipeptidyl transpeptidase enzyme, also known as PC synthase (Zenk 1996). Many heavy metals like Cd, Hg, Cu, Ni, Pb, and Zn cause significant induction of PC biosynthesis but Cd triggers the biosynthesis process more strongly than the others (Zenk 1996). In *Brassica napus*, a sharp increase in PC level was detected following Cd exposure (Carrier and Havaux 2003). Arsenate- and arsenite-induced induction of PC biosynthesis has also been reported (Marcus et al. 2000). Although many heavy metals can trigger the biosynthesis of PCs very few of them (Cd, Hg, Pb, Cu, and Ag) can form stable complex with PCs (Bertrand and Guary 2002). PCs form stable complex with Cd^{2+} ions through the thiolic group (–SH) of Cys and the resulting PC-Cd complexes are transported in the vacuole through the activity of ABC transporters, thus limiting the circulation of free Cd^{2+} inside the cytosol (Sanita Di Toppi and Gabbrielli 1999). Continuous research efforts have been given in recent past to identify and characterize the gene encoding the PC biosynthesis gene PC synthase in angiosperms and Clemens et al. (1999) and Ha et al. (1999) have successfully identified and characterized the structural genes of this important enzyme in *Arabidopsis thaliana, Triticum aestivum*, and *Saccharomyces cerevisiae*.

4.2 Metallothioneins

Metallothioneins (MTs) are ubiquitous, heavy metal-binding, Cys-rich, low-molecular-weight (5–20 kDa), and gene-encoded proteins that can form metal-thiolate clusters via mercaptide bonds (Hamer 1986). Plenty of MT genes have been identified from a wide variety of organisms including prokaryotes (bacteria, fungi) and eukaryotes (animals and plants) (Robinson et al. 1993). Specific physiological functions of MTs have not been described properly but MTs are possibly involved in maintaining homeostasis of essential transition heavy metals mainly Cu sequestration of toxic heavy metals and they may also be involved in developing protection against oxidative damage. Biosynthesis of MTs is transcriptionally regulated and is positively regulated by various factors including hormones, cytotoxic agents, and heavy metals such as Cd, Zn, Hg, Cu, Au, Ag, Co, Ni, and Bi (Yang et al. 2005; Kagi 1991). MTs show maximum affinity for binding with Cu and their expression is also induced when exposed to elevated concentration of copper (Murphy et al. 1997).

5 Phytotoxicity Mechanisms

5.1 Denaturation of Proteins and Inhibition of Enzyme Activity

Different heavy metals including Cd, Ni, and Pb significantly affect the cytosolic protein pool when present in an elevated concentration within the cell. Previous reports have shown reduction in protein level in different plant species under Pb and Ni treatment (Mishra et al. 2006). Ni promoted the denaturation of low-molecular-weight proteins like glutathione. Along with Ni, Cd also disrupts various protein structures by binding to the thiol groups and thus dismantling their structure and inhibiting their activities. Reduced protein content due to heavy metal stress could be due to altered gene expression, enhanced protease activity, and depleted free amino acids due to disrupted nitrogen metabolism (Seregin and Ivanov 2001; Kovalchuk et al. 2005; Gopal and Rizvi 2008; Gajewska et al. 2009).

Besides various proteins, enzymes are among the major targets of different heavy metal ions. Different heavy metals can act as cofactors and increase the efficiency of enzyme activity. But excess heavy metal exposure can be toxic to the enzymes. There are various mechanisms by which heavy metals mainly Pb, Cd, and Ni can inhibit the activity of a wide range of enzymes involved in different metabolic pathways. The most important mechanism is interaction of these metal ions with –SH groups present on the enzymes, thus impairing enzyme activity by hiding the catalytically active groups that remain exposed for the proper enzyme activity (Van Assche and Clijsters 1990; Seregin and Ivanov 2001). Inhibition of about hundred enzymes especially RuBisCO and nitrate reductase by interaction with –SH groups has been reported by various studies (Seregin and Ivanov 2001). Considerable inhibition of the nitrate reductase (NR) enzyme activity in leaves was found in the presence of Pb (Burzynski and Grabowski 1984), Cd (Hasan et al. 2008), and Ni (Yusuf et al. 2010). Besides the interaction with SH groups, it has also been demonstrated that –COOH groups blocked by Pb ions may also play a major role in hampering enzyme activity (Sharma and Dubey 2005).

6 Genotoxicity

One of the major direct effects of heavy metal-mediated toxicity in plant cell is the antimitotic effect (Wierzbicka 1994). Pb-induced decrease in mitotic activity in onion root cells was reported way back in 1928 (Hammett 1928). Pb primarily targets the purine, pyrimidine, and other microtubular proteins after entering into the nucleus and resulted in the disruption of DNA synthesis and cell cycle block at G2 phase (Jiang et al. 2001; Siddiqui et al. 2014). Later, several other studies have also supported this observation in other plant species using different heavy metals such as Cd. Bean seedlings under Cd treatment showed clear reduction in cell division associated with extension of cell cycle (Duan and Wang 1995). In addition to hampering mitotic activity, heavy metals like Cd and Pb can induce different chromosomal anomalies. Following treatment with Cd and Pb several chromosomal aberrations, fragmentation, and conglutination along with chromosomal ring formation, sticky chromosomes and anaphase bridges, c-mitosis, micronuclei formation, and disturbed formation of phragmoplast were observed in beans, garlic, and onion root tip cells (Duan and Wang 1995; Zhao and Mo 1997). One possible reason of induction of chromosomal aberrations is heavy metal-mediated disruption of microtubule assembly-disassembly (Fusconi et al. 2006).

Heavy metals can also act as premutagenic agents. They can reach to the nucleus and binding directly to the DNA or –SH groups of proteins causes various kinds of DNA damages including DNA base modifications, inter- and intramolecular crosslinking of DNA and proteins, DNA strand breaks, rearrangements, and depurination. Several lines of evidences have suggested the link between heavy metal-induced oxidative stress and genotoxicity. Chemical reactions driving these DNA damages are characteristics of an oxidative DNA attack (Kasprzak 1995).

7 Disruption of Photosynthesis

Elevated levels of heavy metals including Ni, Cd, and Pb generally hamper the photosynthetic process due to their direct effect on the photosynthetic apparatus, including thylakoids.

Cd, Ni, and Pb can alter the lipid composition of thylakoid membrane causing disruption of light harvesting complex and photosystem II (PS II). This causes impairment of electron transport associated with photosystem II. Moreover, some heavy metals can replace the central Mg in chlorophyll (Chl). Under heavy metal-stress conditions, reduction in chlorophyll production can be observed. This may be a possible consequence of heavy metal-mediated inhibition of the activity of the enzymes associated with chlorophyll biosynthetic pathway. Decrease in chlorophyll content is one of the major effects of Cd-, Pb-, and Ni-mediated toxicity (Ewais 1997; Pandey and Sharma 2002; Gopal and Rizvi 2008). Along with the inhibition of chlorophyll-synthesizing enzymes, heavy metal-mediated promotion of chlorophyllase activity may also contribute to the decreased chlorophyll content (Drazkiewicz 1994; Abdel-Basset et al. 1995). In addition, Ni, Cd, and Pb can also affect the carbon fixation pathway by inhibiting the activities of the Calvin cycle enzymes such as RuBisCO (Seregin and Ivanov 2001). Inhibition of Calvin cycle reactions leads to the accumulation of ATP and NADPH produced during Z-scheme of light reactions, resulting in a high pH gradient across the thylakoid membrane, which hampers the PSII activity (Krupa and Baszynski 1995). Another additional mechanism contributing to the disruption of the photosynthetic efficiency is the induction of stomatal closure in heavy metal-exposed plants that limits CO_2 uptake by plants for carbon assimilation (Parys et al. 1998). It is also important to note that heavy metals also perturb both carbohydrate metabolism and transport. Inhibition of activity of α-amylase, β-amylase, and sucrose phosphate synthase with the concomitant increase in the activity of starch phosphorylase, acid invertase, and sucrose synthase enzyme causes accumulation of carbohydrates in the leaves (Jha and Dubey 2004).

The molecular basis of all these alterations in photosynthetic process is not much revealed but these may be the results of elevated level of reactive oxygen species (ROS) and oxidative stress due to heavy metal toxicity.

8 Heavy Metal-Mediated ROS Production

One of the earliest deleterious effects of heavy metals in higher plants is overproduction of ROS resulting in oxidative stress (Fig. 2). Cellular organelles like chlorophyll, mitochondria, and peroxisome where oxidizing metabolic activity is significantly high and electron flow occurs are the main source of ROS production in plant cells (Apel and Hirt 2004; Mittler et al. 2004). On the basis of redox activity, heavy metals can be divided into two groups: redox inactive (Cd, Zn, Ni, Al, etc.) and redox active (Fe, Cu, Cr, Co, etc.) (Dietz et al. 1999; Schutzendubel and Polle 2002). Redox-active heavy metals are directly involved in redox reactions, resulting in the production of different kinds of ROS in the cell. On the other hand, redox-inactive heavy metals cannot efficiently catalyze the redox reactions to favor

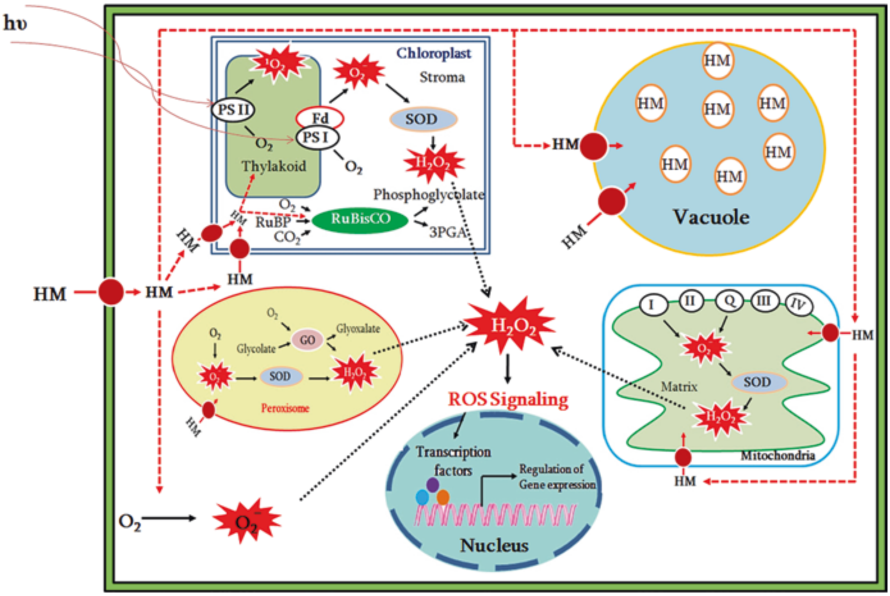

Fig. 2 Heavy metal-mediated ROS production in different organelles including mitochondria, chloroplast, and peroxisome and activation of ROS signaling

ROS production directly; rather they can indirectly boost up ROS generation in several ways including interaction with antioxidant defense system, affecting the electron transport system or elevating lipid peroxidation process in different cell organelles.

Different heavy metals, especially Cd, hamper the H_2O system by replacing the central Mg atom with Ca^{2+} or Mn^{2+}, resulting in uncoupling of electron transport in the PSII. Cd is also involved in inhibiting the electron flow in the reducing side of PSI. Along with this, heavy metals also exert negative effect on the carboxylation phase of photosynthesis. Heavy metals negatively affect the activity of two key enzymes of the carbon fixation reaction including RuBisCO and phosphoenolpyruvate carboxylase (PEPC). Cd^{2+} can hamper the activity of RuBisCO by disrupting its structure. It does so by displacing the central Mg^{2+} ion which acts as a crucial cofactor for the carboxylase activity of the enzyme. This may shift the activity of RuBisCO towards oxygenation reactions (Krantev et al. 2008; Siedlecka and BaszynAski 1993; Siedlecka et al. 1998), which results in the production of glycolate. The latter then moves to peroxisome, gets oxidized by glycolate oxidase, and produces H_2O_2. Along with glycolate oxidase, many other enzymes including glycolate oxidase, flavin oxidase, and xanthine oxidase are involved in the production of different types of ROS such as H_2O_2 and $O_2^{\bullet-}$. Different heavy metals can modulate the peroxisomal mobility resulting in enhanced production of different reactive oxygen species. In mitochondria, the main site of ROS production is complex I and the ubiquinone zone, where Q-cycle occurs. Heavy metal cations can significantly affect the mitochondrial electron transport system resulting in induction of oxidative stress. Heavy metals can accelerate NADPH oxidase (NOX)-mediated ROS production, in which NOX mediates the movement of electrons from cytosolic NADPH to O_2 resulting in the formation of $O_2^{\bullet-}$. NOX-mediated acceleration in ROS production has been reported under different heavy metal stresses including Ni, Pb, Cd, and Cu in different plant species like *Pisum sativum*, *Vicia faba*, and *Arabidopsis thaliana* (Hao et al. 2006; Rodríguez-Serrano et al. 2006; Pourrut et al. 2008).

In addition to the induction of metabolic ROS production, the redox-active transition heavy metals (Fe, Cu, Cr, V, etc.) are directly involved in ROS production through Haber-Weiss and Fenton reaction (Halliwell 2006). Transition metals like Cu and Fe possess unpaired electrons, which act as potential catalysts to reduce O_2 resulting in the formation of $O_2^{\bullet-}$ in the following reaction:

$$M^n + O_2 \rightarrow M^{n+1} + O_2^-$$

This $O_2^{\bullet-}$ is subsequently converted to H_2O_2 under neutral pH conditions. H_2O_2 is further decomposed to form OH. If the transition metal is Cu, the name of the reaction is Haber-Weiss reaction and in case of Fe, this is known as Fenton reaction:

$$M^{n+1} + O_2^- \rightarrow M^n + O_2$$

$$M^{n} + H_2O_2 \rightarrow M^{n+1} + \cdot OH^{-} + \cdot OH$$

Immediately after their production, reactive oxygen species (ROS) are generally scavenged at their sites of production by the antioxidants present in the cell. However, when this local antioxidant system becomes unable to cope up with excessive ROS generation, H_2O_2 can leak in the cytosol and diffuse to other compartments and develop oxidative stress which hampers various cellular activities. The elevated accumulation of ROS inside cytosol can damage the cellular structures and biological macromolecules including membranes, proteins, lipids, and nucleic acids resulting in lipid peroxidation (Foyer and Noctor 2005).

9 Plant Signaling in Response to Heavy Metal Stress

9.1 MAPK Signaling

MAPKs are very much crucial and evolutionarily conserved signaling molecules, which play a vital role in various abiotic stress responses in plants. Among various abiotic stress factors, heavy metals can stimulate this signaling process and are activated either directly by specific metal ligand or indirectly by the ROS produced due to heavy metals. Previous several studies have demonstrated the activation of MAPK signaling cascade in response to various heavy metals including Cd, Cu, and As (Jonak et al. 2004; Yeh et al. 2007; Rao et al. 2011; Smeets et al. 2013). In *Arabidopsis*, two well-known MAPKs, MAPK3 and MAPK6, are induced by $CdCl_2$ and $CuSO_4$ (Pitzschke et al. 2009; Liu et al. 2010; Takahashi et al. 2011; Sethi et al. 2014). Like *Arabidopsis*, in rice, expression of MAPK gene OsMAPK2 and several MAPK gene homologs including OsMSRMK2, OsMSRMK3, and OsWJUMK1 increased considerably under Cd and Cu stress in leaves and roots (Yeh et al. 2004, 2007; Rao et al. 2011). Besides this, elevated level of Cd and Cu can activate MAPK signaling in Medicago (Jonak et al. 2004). This heavy metal-mediated activation of MAPK signaling cascade is probably due to ROS generation, accumulation, and alteration in antioxidant system.

9.2 Calcium Signaling

Calcium ions (Ca^{2+}) act as universal secondary messengers and are associated with normal functioning of the cell. Cytosolic Ca^{2+} concentration changes in response to various environmental stimuli including heavy metal stress. The transient increase in cytosolic Ca^{2+} concentration is sensed by a diverse group of Ca^{2+} sensors including calmodulins (CaMs), CaM-like proteins, calcineurin B-like proteins (CBLs), and Ca2C-dependent protein kinases (CDPKs) (Conde et al. 2011; Steinhorst and

Kudla 2014). Transcript profiling study in roots of rice showed increment in the activity of CDPKs with increasing concentration of Cr (IV) indicating involvement of these proteins in mediating the Cr stress response (Huang et al. 2014). Another study in foxtail millet indicated that Ca^{2+} signaling in association with hydrogen sulfide imparted improved tolerance to Cr (IV)-mediated heavy metal stress (Zhao et al. 2015). Expression pattern of calmodulins, another important Ca^{2+} sensor, under arsenic stress indicates the possible involvements of different Ca^{2+} signaling components in mediating the stress response (Chakrabarty et al. 2009).

9.3 Hormone Signaling

Auxin is one of the important phytohormones involved in developmental as well as environmental stress responses. In response to metal stress, auxin homeostasis is modulated. Some recent studies have shown that under heavy metal stress, location and deposition of auxin are regulated by differential expression of auxin-related genes including phosphoribosyl anthranilate transferase 1 (PAT1), CYP79B2 and CYP79B3, YUCCA (YUC), Gretchen Hagen (GH3) genes, (TIR1), PIN family, and ABCB family (Wang et al. 2015). Another study demonstrated that excess Cu^{2+} level hampered both auxin and cytokinin accumulations and mitotic activity in primary and secondary root tip cells (Lequeux et al. 2010).

10 Conclusion and Future Perspective

Heavy metal contamination and toxicity due to increasing anthropogenic activity have become a major concern and a global issue in the last decade. Many heavy metals persist in the soil for many years due to their nonbiodegradable nature and enter into the food chain mainly through the root system of plants, thus causing deleterious health effects. Heavy metal ions take entry into the plant root cells through a number of transporters present in the membrane. In plants, these ions may accumulate within various subcellular compartments of root cells, mainly vacuole and Golgi apparatus, or may be transported to different parts of the shoot via the transpiration stream. Inside plant cell, metal ions interact with different biomolecules and thus affect several physiological as well as metabolic processes including photosynthesis and respiration.

Although significant developments have taken place in recent past to understand the mechanism of heavy metal-mediated phytotoxicity and detoxification, many crucial components of this complex network still remain unidentified. Especially knowledge on the signaling molecules of the metal-induced signal transduction, including proper sensing of the change in heavy metal concentration and subsequent activation of downstream regulation of several genes at the transcription level, remains largely illusive. So, the extensive understanding of heavy metal-mediated signaling pathways and their cross talks remain as a future perspective. As cellular and molecular mechanism of heavy metal toxicity and subsequent stress response developed by plant cells appear to be necessary to understand the impact of heavy metal toxicity, as well as to improve plant heavy metal tolerance that ultimately reduces the chance of entering heavy metal into the food chain, much work is needed to fully unveil the mechanisms associated with toxicity of heavy metals in plants as well as the mechanisms associated with plant metal tolerance.

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Heavy Metal Toxicity, Mechanism, and Regulation

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1 Introduction

Metal toxicity is a global problem for the environment, agricultural activities, and health. After putting in strong efforts towards purification of water, plants have been the "green" movement starting from the 1970s to 1990s. Many "Western" countries consider metal toxicity as a problem of the "developing" and "transition economy" of the countries. However, this is not a correct norm, as the richest of the countries and developed ones still have metal toxicity as a concern for plants and the environment in general. Chemicals such as chromium, cadmium, arsenic, and lead are heavy metals (HM) which are produced by various industries. The chemicals from the industries seep deep into the land, causing groundwater and soil pollution. This groundwater is then used to grow staple crops such as rice, wheat, maize, and millet, thus leading to uptake of heavy metals and causing toxicity in plants (Dubey et al. 2010) (Fig. 1).

Strict compartmentation and chelation have to be maintained all the time for a balanced cytosolic metal concentration. The vacuole in plant cells is the main storage compartmentation site for heavy metals. Plants have several chelating molecules to sequester the metal toxicity to an acceptable level. These are phytochelatins (PCs) and metallothioneins. Leaves from barley, grown in the heavy metal-polluted environment, showed that cadmium (Cd), molybdenum (Mo), and zinc (Zc) are mainly subjected to vacuolar sequestration. As the HM toxicity crosses a threshold, it stimulates the expression of reactive oxygen species producing enzymes which synthesize more ROS (Dubey et al. 2018a), which further degrades the cell structure and

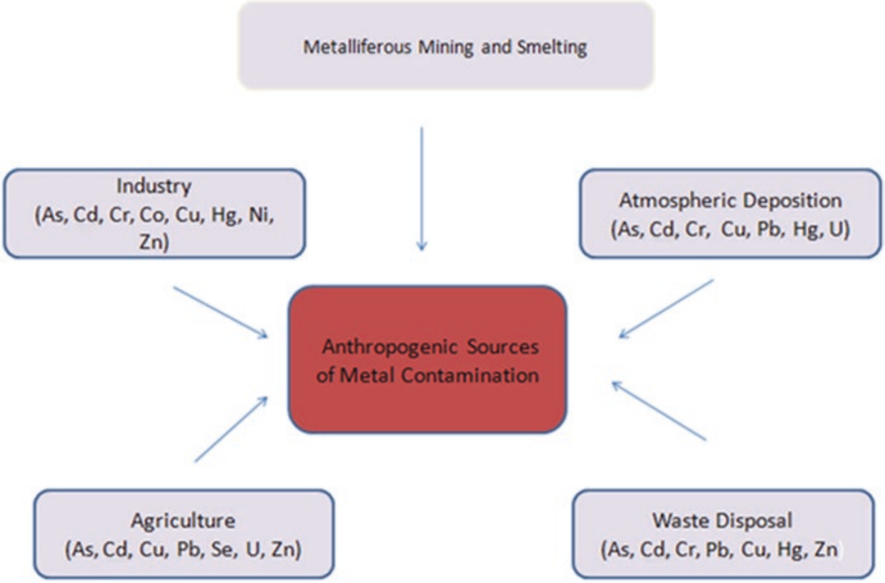

Fig. 1 Anthropogenic activities in heavy metal contamination

function. Metal toxicity also stimulates defense signaling cascades and expression of small noncoding RNAs such as miRNA. miRNAs are ~22-nucleotide, endogenously expressed RNAs that bind with 3′UTR of mRNA and regulate the gene expression at posttranscriptional level by either degradation or translational repression of mRNA. Stress conditions are also known to influence secondary metabolites in plants. Shikimate phenylpropanoid biosynthetic pathway in plants produces the most significant secondary metabolites, i.e., phenolic acids and flavonoids. These are considered as the biomarkers of biotic and abiotic stress tolerance in plants. Under stress, the production of phenolic increases as compared to non-stressed plants, as these compounds are said to be involved in oxidative stress caused by ROS. Similarly, increase in the production of flavonoids during heavy metal stress is due to its possible action as metal chelator and as antioxidants to protect the plants against the oxidative stress by high levels of heavy metals (Dubey et al. 2010, 2018a). Negative impacts of heavy metals depend on several factors including metal type, its concentration, its oxidation state, and its duration.

2 Uptake and Accumulation

2.1 Mechanism of Heavy Metal Uptake

There is a very little amount of organic arsenic present in the soil along with arsenate [As(V)] and arsenite [As (III)] which are predominantly present in the environment. As(V) enters the root cells via high-affinity phosphate transport system as it is a phosphate analog. As (III) transport takes place via membranes of the silicon transporter nodulin 26-like intrinsic protein because As(III) shows similarities with silicic acid.

Cadmium (Cd) transport involves the ZRT- and IRT-like protein family transporters which are responsible for the transportation of Fe, Zn, and Mn. Cadmium uptake is also mediated by natural resistance-associated macrophage protein (NRAMP) family. In rice, *Oryza sativa* OsNramp1, which is an iron transporter located at the plasma membrane, has been shown to support Cd influx.

Lead (Pb) is one of the most hazardous heavy metals which causes pollution of water bodies. Lead directly adheres to the polysaccharides of the rhizodermis cell surface or gets bound to the carboxyl groups of mucilage uronic acid (Dubey et al. 2018b). Mainly, Pb enters the plants cells through ionic pathways although it may enter through several pathways which are still unidentified.

Chromium is nonessential for plants and its uptake is both active and passive, but majorly active pathway, as its translocation is aided by various membrane-based transport proteins, which often act as carriers of different anions present in the root epidermis. This makes Cr to compete with essential ions like Fe, S, and P for uptake, leading to a competitive hindrance to the metals by reducing their uptake rate (Jaishankar et al. 2014).

2.2 Effects on Growth and Development of Plant

In the environment, heavy metal can be found, which leads to some changes in the growth and development of plants. The effects of these heavy metals' toxicity are seen with the naked eye as atrophied growth, and leaf blight and ripple, showing high ROS production, and some other effects are found in the genotype that triggers the activation of the stress response genes and results in the generation of ROS (Tables 1 and 2) (Connett and Wetterhahn 1986; Mishra et al. 2017).

2.3 Physical Effects on Plants

Table 1 Physical effects in heavy metal-stressed plants

Process	Effect in heavy metal stressed plants
Germination	• Reduced germination rate • Less yield
Root	• Decrease in root length • Reduced dry weight of plant • Elongated root hairs
Shoot	• Reduced Plant height
Leaf growth	• Reduced foliage area • Leaf yellowing starts followed by curling
Yield	• Reduced number of plantlets • Stunned growth and reduced biomass

2.4 Effects on Physiological Processes

Table 2 Physiological effects on heavy metal-stressed plants

Process	Effects on heavy metal-stressed plants
Photosynthesis	• Inhibition of ETS • Reduced CO_2 fixation • Deregulation of chloroplast function
Water relations	• Reduction in water potential • Elevated rate of transpiration
Mineral nutrition	• Reduced uptake of Fe, S, and P
Enzymatic contents	• Upregulation of stress-responsive enzymes • Increased ROS scavenging enzymes

3 Plant Response to Heavy Metal Stress

1. Immobilization of HM, as to escape its routes to the vegetative plants
2. Compartmentalization of HM
3. Exclusion of uptake from soil
4. Chelation

3.1 Biochemical Response

- Upon induction of HM stress the probable enzymatic modifications in plants
- Reduced pigment production like chlorophyll and anthocyanin
- HM stress-induced upregulation in the production of metabolites (e.g., glutathione, ascorbic acid) causing damage to the plants (Gratão et al. 2005)
- Alterations in the levels of compounds (stress responsive) which confer heavy metal tolerance resistance in plants to stress (e.g., phytochelatin, histidine)

4 Effects on Antioxidant Enzyme

4.1 Glutathione

Based on the previous studies we know that the level of GSH synthesis has been found to be downregulated in heavy metal stress. The low level is observed mainly in roots under different metal stress conditions, which indicates the role of stress response induced by As, Cr, Cd, and Cu. Where these ions react with sulfhydryl group of GSH it becomes unstable (Schützendübel and Polle 2002; Tangahu et al. 2011). Certain loss can be seen in the pool of GSH which is accounted by complex formation of N-acetylcysteine, γ-glutamylcystein, and glutathione-XO3-thiolate. Due to these complex formation and ROS upregulation, lipid membrane of cell is modulated (Zhou et al. 2014; Patra et al. 2004).

4.2 Role of Phytochelatins

Phytochelatins can be found in the tissues of plants and cell cultures. The essential metal can be detected in quantity and correlated with the help of metal ion depletion of the medium; we can observe the level of phytochelatins in cell cultures (Patra et al. 2004; Zengin and Munzuroglu 2005). These observations conclude a role of phytochelatins in the metabolism of essential metal ion through homeostasis. They also abduct themselves in vacuole. Several transcriptome studies conclude the mechanism of self-destruction, which is reported only for certain heavy metals, such as for remediation of Pb, Cu, and Cd (Hall 2002).

5 Plant Signaling in Response to Heavy Metals

The inability of plants to escape from environmental stresses such as metal pollution has driven the evolution of multiple mechanisms to efficiently sense, respond, and therefore adapt to such stresses. Sensing of heavy metals by plants generates a response such as modulation of molecular and biochemical mechanisms of cell. Certainly, this response is evoked by important signal transduction network operated in plant cells formed by several signal transduction units (Hossain et al. 2012; Farid et al. 2013). The ultimate response of plant is shown by synthesizing metal transporter proteins and metal-binding proteins helping the plant to counteract excessive metal stress.

5.1 MAPK Signaling in Heavy Metal Stress

Mitogen-activated protein kinase (MAPK) is one of the most important and highly conserved signaling molecules that function in response to many diverse stresses and during many developmental pathways. MAPK cascade consists of three tier components MAPKKKs, MAPKKs, and MAPKs mediating phosphorylation reactions from upstream receptor to downstream target. MAPK signaling mediates the transmission of stress-related signals, thus regulating large number of cellular processes (Dubey et al. 2010; Sytar et al. 2013). Among abiotic stresses, heavy metal stress has conferred profound effect on MAPK signaling pathways. MAPKs are known to be activated by perception of specific metal ligand and also by ROS molecules produced in the metal stress (Rellán-Álvarez et al. 2006; Hatata and Abdel-Aal 2008) (Fig. 2).

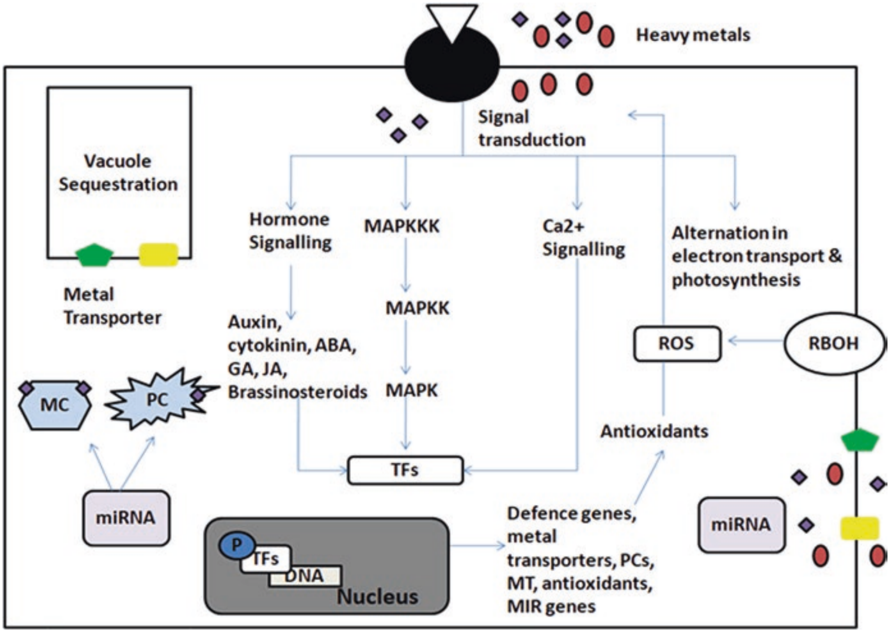

Fig. 2 Cross-linking of signaling pathways and its ultimate response in heavy metal stress. This figure displays the involvement of several signaling components working during metal stress. Sensing of significant level of heavy metals by plants initiates signaling network causing activation of various metal-responsive transcription factors. These transcription factors regulate the expression of metal-responsive and other stress-related genes ultimately helping the plant to counteract stressed situation. These stress-related genes are mainly metal transporters, phytochelatins, metallothionine, antioxidant genes, and miRNA (MIR genes)

5.2 Calcium Signaling in Heavy Metal Stress

The calcium ion (Ca2+) as supported by different studies acts as a universal secondary messenger in the normal functioning of plants as well as in response to various environmental stresses. The cytosolic free Ca2+ concentration changes in response to various metal stress stimuli triggering complex interactions and signal transduction pathways (Dubey et al. 2010). This transient increase in the cytosolic concentration is perceived by highly sensitive calcium-sensing proteins that mediate this chemical signal into a biological response. Plants harbor myriads of calcium-sensing proteins such as calmodulins (CaMs), CaM-like proteins (CMLs), calcineurin B-like proteins (CBLs), and Ca2+-dependent protein kinases (CDPKs) that bind to Ca2+ and trigger different downstream signaling pathways.

5.3 Hormone Signaling

Ethylene (ET) is biosynthesized by ACC synthase (ACCS) that converts AdoMet to ACC, while ACC oxidase (ACCO) catalyzes the conversion of ACC to ethylene. The consequences of metal stress on ethylene production in plants are both metal and concentration dependent. Major five ET synthesis genes from rice OsACS2, OsACO1, OsACO2, OsACO5, and OsACO6 alongside transcription factors AP2 and ERF1 from alfalfa were found to be upregulated in Hg treatment (Dubey et al. 2010, 2018a; Hatata and Abdel-Aal 2008; Prasad 2004). However, in rice, genes involved in cytokinin signaling (OsRR1, 3, 4, 6, and 11) were downregulated, suggesting that both ET and CK may regulate the Hg-induced inhibition of rice root growth. Recently, it had been revealed that in wheat ET negatively regulates Al-induced efflux of malate ions using ET8, which is a crucial mechanism for Al tolerance. Cytokinins (CKs) are N6-prenylated adenine derivatives involved within the regulation of plant growth and development and in biotic and abiotic stresses. There are reports of CKs in plants activated upon heavy metal stress that are ready to alleviate heavy metal-induced toxicity (Manara 2012; Valko et al. 2005) (Fig. 3).

6 Regulation of microRNAs During Heavy Metal Stress

Besides the contribution of signaling pathways in transmitting heavy metal-related stimuli and regulating the plant response, other regulators like small RNAs are majorly found to have profound effect on metal stress response. Small RNAs such as microRNAs are a 20–24-nucleotide noncoding RNAs that regulate the gene expression at posttranscriptional level by targeting mRNA degradation or by translation repression. It has been shown that different miRNA families are differentially regulated temporally as well as spatially, differing in concentration from species to species (Jozefczak et al. 2012). All these data indicate that differential regulation of any miRNA depends upon the function of miRNA target, physiology, and metabolism of the plant. Recently genome-wide transcriptome analysis and high-throughput sequencing have been used to identify microRNAs, which are responsive to heavy metal stress in many plant species (Flora 2009; Bielen et al. 2013). It has been shown that various conserved miRNAs are differentially regulated during the normal and stress conditions. Differential expression of miRNAs in heavy metal stress indicates the possible involvement of miRNAs in heavy metal stress detoxification and tolerance. In the next section, we will discuss about the tools and databases which can be used to identify and characterize microRNAs reported during various stresses (Table 3).

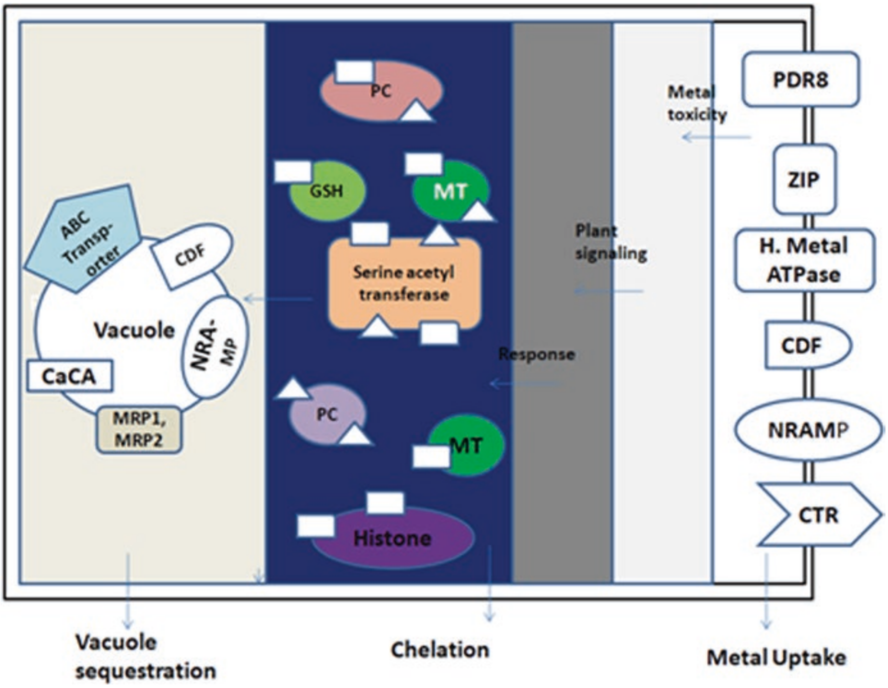

Fig. 3 Metal detection, plant signaling, and sequestration. Different transporters are involved in metal ion uptake. Elevated level of these heavy metals triggers different signaling modules which transmit the signals inside cell, thus triggering defense response. The toxicity of these metals inside the cell is sequestrated by metal chelators like phytochelatins and metallothioneins. The chelated metals are then ultimately transported to the vacuoles with the help of metal transporters present on the vacuole membrane

6.1 PASmiRbase Databases

Establishment of the PASmiR database necessitated the development of a curated standard nomenclature for miRNAs and abiotic stresses. Each miRNA inputted into the PASmiR database is converted to a standard terminology by referring to miRBase as follows: a prefix of species abbreviation based on Latin name is followed by a dash, “miR,” and a family number. For example, “osa-miR172” is the correct terminology for *Oryza sativa* miRNA from family 172.

For the purposes of inclusion in PASmiR, each inputted abiotic stress is assigned unique names and manually classified into one of 11 abiotic stress classes (drought, salinity, high temperature, HM toxicity, etc.) according to physiological affection (Gajalakshmi et al. 2012; Hawley et al. 2004).

Table 3 Various tools to characterize microRNA

S. no.	Method	Feature
1	Target Scan	Vertebrate-conserved microRNA target database
2	MiRanda	Sequence complementarity based on position-specific rules and interspecies conservation is optimized
3	InMiR	Using liner Gaussian model provides a dataset of 1935 predicted mRNA targets for 22 intronic miRNAs
4	Pic Tar	Details about 3′ UTR alignments with predicted sites and links to various public databases are provided
5	Star Base	Database with intersections among targets by predictive software
6	RNA-hybrid	Establishes the most favorable hybridization site between two sequences
7	miRTar	Relationships between a group of known/putative miRNAs and protein-coding genes are identified
8	psRNA target	MiRNA and target mRNA reverse complementary matching using a proven scoring schema and calculating unpaired energy required to open secondary structure around the miRNA Target site on mRNA to evaluate target-site accessibility

6.2 miRBase

In bioinformatics, miRBase is a biological database that acts as an archive of microRNA sequence and annotation.

miRBase has five aims:

1. To provide a consistent naming system for microRNAs
2. To provide a central place collecting all known microRNA sequences
3. To provide human and computer readable information for each microRNA
4. To provide primary evidence for each microRNA
5. To aggregate and link to microRNA target information

miRbase contains miRNAs belonging to various species belonging to Alveolata, Chromalveolata, Metazoan, Mycetozoa, Viridiplantae, and Viruses. For Viridiplantae, in release 21 (2014) data is available for 73 species. This includes 4800 unique mature miRNAs and 8480 precursor sequences. Further for the target prediction of identified microRNAs, plant-specific target prediction tool psRNA is used (Oliveira 2012).

6.3 psRNA Target Prediction Tool

psRNA Target is a web server for plant small RNA target analysis. The psRNA target is based on two important features: miRNA and target mRNA reverse complementary matching using a proven scoring schema and calculating unpaired energy required to open secondary structure around the miRNA target site on mRNA to evaluate target-site accessibility.

By implementing a distributed computing pipeline, it is designed for high-throughput analysis of next-generation data which runs on a Linux cluster at back end (Dalvi and Bhalerao 2013). Front end includes three user-friendly interfaces to accept user-submitted small RNAs and transcript sequences and outputs a detailed list of small RNAs and matching target sites (Flora 2009).

6.4 Plant Regulatory Small RNAs

Plant regulatory small RNAs (sRNAs) are produced from double-stranded RNA duplexes or hairpin single-stranded RNA precursors by the endonuclease activities of Dicer-like (DCL) proteins. sRNAs produced from double-stranded duplexes are referred to as small interfering RNAs (siRNAs), while sRNAs produced from the stem-loops of single-stranded precursors are referred to as microRNAs (miRNAs) (Oliveira 2012). Plant regulatory sRNAs control a range of cellular and developmental functions, including plant cellular defense mechanisms against RNA viruses, transcriptional gene silencing by guiding heterochromatin formation at homologous loci, and sRNA-mediated DNA methylation. Among the many roles of plant sRNAs, posttranscriptional gene silencing and translational inhibition guided by miRNA and phased small interfering RNA (phasiRNA) are the two most widely studied mechanisms, in which miRNA and phasiRNA share the same targeting mechanism.

6.5 Need for the Prediction of miRNA Target for Regulation

There are certain miRNAs which do not code for any molecule, their length is very short about 19–21 nucleotide base pairs as compared to other RNAs, and they bind to mRNA. Their mechanisms are also different from other RNAs because they do not code for protein. Their role is specific; for example it controls the gene expression biologically and also involves in posttranscriptional level. They are found in various organisms of biological classification in which the splicing of RNA can be seen in living organism. The main reason of the mRNA degradation is to control protein translation (Nematshahi et al. 2012; Shanker et al. 2005).

Different gene codes for different mRNAs and miRNAs bind to these targets post-transcriptionally. If miRNAs binits the target messenger RNA (3′ untranslated region) their nature is functionally negative. One should study the regulation of different miRNAs to understand the various functions of our cellular organisms like the proliferation of cell and their metabolism, and death. There are various methods in which we can identify the role of miRNA in plants and animals. With the help of data sequencing we can identify the novel and conserved miRNA and after that we can find the target of particular miRNA.

6.6 microRNAs Under Plant Stress

Several common microRNAs are reported in literature for their role in regulation of stress-related response under different abiotic stresses mainly heavy metal toxicity that are miR156. miR157, miR158, miR159, miR161, miR62, miR165, miR66, miR168, miR169, miR171, miR393, miR397, and miR408 showed extraordinarily high expression where miR158 had the most abundant expression level to characterize differential expression of genes in plants under stress. The moderately abundant miRNA targets were miR164, miR167, miR319, miR400, miR825, miR827, miR854, miR860, and miR1885 had moderately low abundance. Lowest abundance was showed by miR535, miR824, miR845, miR858, miR7767, and miR2111 miRNAs. Thus effective study regarding miRNA regulation under stress may be of great advantage, thus mimicking the regulation of miRNA and differential expression of genes.

7 Conclusion

From this chapter, we imply that heavy metals show various effects on plants through functional modulation at various levels. Resistance against other environmental stresses is also lost under metal stresses due to activation of signaling pathways. Many of the transcription factors and cytosolic proteins are functionally activated due to activation of MAPK signaling pathways that are responsible in managing stress.

In this chapter we have reported the impact of heavy metals on activation of hormone, calcium, and MAPK signaling along with some transcriptional regulators like transcription factors and miRNAs.

MAPK signaling plays an important role in regulating and combating against heavy metal stress and thus also interplays with calcium, auxin, and ethylene signaling in response to heavy metal stress. Elaborative study regarding animal response towards heavy metal stress is done and thus plant study is of advantageous importance to study signaling regulation in plants.

Additionally, other regulators like miRNAs and transcription factors show response to heavy metal stress. Certain mechanisms up- or downregulate miRNAs which show differential expression of characterized genes. However, the fragmentary work performed keeps this area mysterious in plants. Exploring mechanism and regulators of these metal transporters will contribute significantly in unraveling the mechanisms of metal stress tolerance in plants.

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Effect of Heavy Metal Stress on Growth and Yields of Crop Plants: An Overview

Zahir Muhammad, Abdul Majeed, Rehmanullah, Naila Inayat, and Saira Siyar

Contents

1 Introduction

Elements which are denser than water and possess specific gravity >5 are generally regarded as heavy metals (HM) (Shahid et al. 2015; Dubey et al. 2018). Reportedly, Zn, Pb, Hg, Cd, Ni, and Cu are among the most occurring HM in most of the soils (Belimov et al. 2003). Although some HM are necessary in small amounts for plants and soil microbes, in larger quantities however, most of the HM serve as pollutants and are toxic to living communities (Rout and Das 2002). Industrial activities and agricultural intensification are the leading anthropogenic sources of heavy metal contamination in cultivated soils throughout the world (Edelstein and Ben-Hur 2018). Soils polluted with the occurrence of heavy metals are emerging threats to the rhizospheric communities of living organisms, ecosystem stability, and the

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environment. Since proper growth and physiological functions of plants depend on suitable soil types and prevailing nutrient availability, heavy metal-contaminated soils may create a stressed environment unsuitable for most of the plant species.

Crop plants play an important role in addressing global food demands by providing raw as well as processed materials for human and animal feed. In the current scenario of growing human being population, their sustainable production is necessary which requires enormous improvement in cultivated soils and agricultural practices. To produce more crops for meeting future demands, greater exertions have been made in the agricultural sector during the past few decades throughout the world which though has enormously reduced the gap between crop production and their consumption but at the cost of the ecosystem and environmental degradations (Majeed et al. 2019). Extensive agricultural practices such as the use of agrochemicals, poor irrigation, overcultivation of a soil with a specific crop type, and industrial and mining activities have deteriorated the quality of soils in most of the world by adding heavy metals and other pollutants to them (Nagajyoti et al. 2010; Khan et al. 2015).

Soils contaminated with heavy metals are not ideal for growth and establishment of crop plants because in such soils the fate of nutrients and water remain uncertain due to chemical interaction with HM. Furthermore, beneficial microorganisms to crop plants in soils may be adversely affected both in population structure and in function by the occurrence of heavy metals. Overall results will become evident in poor germination, growth, and physiological functions of plants cultivated on heavy metal-infested soils. Heavy metals particularly those which are nonessential for plants' growth are toxic beyond tolerable levels and may cause oxidative stress, abnormal mineral uptake by plant roots, cellular damage, enzymatic abnormalities, malfunctions of photosynthetic systems, chlorosis, blockage of necessary biomolecules, and reduced biomass, growth, and yields (Dixit et al. 2001; Chaoui and El Ferjani 2005; Nagajyoti et al. 2010). In many experiments, reduced plant growth, biomass, photosynthesis, pigment concentrations, transpiration, and nutrient uptake were documented in *Pisum sativum* (Sandalio et al. 2001; Majeed et al. 2019), maize (Mahmood et al. 2005), *Brassica juncea* (John et al. 2009), wheat (Gang et al. 2013), *Crambe abyssinica* (Hu et al. 2015), lettuce (Silva et al. 2017), and many other plants which were exposed to different heavy metals.

To reduce the adverse effect of heavy metals on plants in general while cultivated crops in specific, different techniques are employed. Based on the concentration of heavy metals and soil types various methods such as physical and chemical reclamation (Dermont et al. 2008), use of microorganisms (Ayangbenro and Babalola 2017), phytoremediation by utilizing plant species (Sarwar et al. 2017), and cultivating of genetically modified plants on polluted soils (Kotrba et al. 2009) have been practiced to remove heavy metals from soils and alleviate their drastic effects on plants. In this chapter, we first discuss the toxic effects of heavy metals on crop plants and then highlight sustainable approaches for managing the problem of heavy metal contamination.

2 Responses of Crop Plants to Heavy Metal Stress

Essential metals like Zn, Cu, Mo, Mn, Co, and Ni in excessive amounts while non-essential heavy metals (e.g., As, Cr, Ag, Cd, Pb, Se, Hg) in fewer quantities have toxic effects on plants (Sarwar et al. 2017; Tiwari and Lata 2018). Different plants exhibit different responses to heavy metal stress; however, types of heavy metals and their concentration are the leading determinants in expressing such toxicity. Although some plants may be relatively tolerant to metal stress, in general nearly all the growth, developmental, and physiological phenomena of most of the plants are prone to toxic consequences of heavy metal stress which result in lower yield and production in case of crop plants. Poor germination, reduced growth of root and shoot, nutrient uptake, and several metabolic activities of plants can be adversely affected by heavy metals as a result of oxidative stress, enzyme disruption, and abnormalities in membranal transporters. Table 1 illustrates the effect of different heavy metals on different crop plants.

2.1 Effect on Germination

Germination of seeds is an important event in the life cycle of crop plants whose proper initiation and proceeding ensure the vital establishment, growth, and production of crops. Hindering factors acting against proper germination procedures result in either complete germination arrest or poor germination which leads to reduced growth and physiological activities of the seedling. Studies have demonstrated that heavy metal stress imparts drastic effects on germination of plants. Li et al. (2005) demonstrated that seed germination of *Arabidopsis thaliana* exposed to Cd, Pb, and Hg showed differential responses to stress. In general, seeds were more sensitive to Hg-exposed stress where germination was completely inhibited although other two metals also restricted germination. Abbas and Ali (2007) revealed that Ag-, Zn-, and Pb-treated mung bean seeds showed differential responses in terms of germination percentage. Zn caused stimulation while Ag and Pb had a detrimental effect on seed germination when their concentration increased. A significant retardation in the germination of sunflower was imparted by Cr at a concentration of 60 mg kg-1 in pot culture studies which demonstrated the toxic behavior of heavy metals (Fozia et al. 2008). In melon seeds, reduced germination, germination index, uniformity, and mean germination time were caused by Cr at a concentration of 300 mg l^{-1} (Akinci and Akinci 2010).

Reduced seed germination in plants as a result of heavy metal stress may partly be attributed to water deficiency in soil and partly to membrane disruption caused by heavy metals. Enzymes which govern the successful accomplishment of germination events may be affected by heavy metal stress resulting in an abnormal pattern of seed germination. It has been suggested that some heavy metals such as Cd may cause reduced activity of ATPase, affect the function of the plasma membrane, and

Table 1 Effect of different heavy metals on germination, growth, and biochemical attributes of some crop plants

Heavy metals	Concentration	Target crop plants	Effect	References
Cd, Pb	50–500 mg l^{-1}	Wheat (*Triticum aestivum*)	Drastic effect on growth and pigments with Cd treatment; Pb did not alter the studied parameters	Öncel et al. (2000)
Zn, Cd, Cr, Ni, Cu	5–40 ppm	Alfalfa (*Medicago sativa*)	Reduced germination, seedling growth	Peralta et al. (2001)
Cd	100 and 500 μM	Rice (*Oryza sativa*)	Oxidative stress induction	Shah et al. (2001)
Co, Cd, Ni	500 μM	Cabbage (*Brassica oleracea*)	Severe abnormalities in growth, physiology, pigments	Pandey and Sharma (2002)
Pb, Zn, Ni, Cu	10 μM	Maize (*Zea mays*)	Suppression of seedling growth at higher concentration	Bashmakov et al. (2005)
Ni	1.0, 10, and 100 μM	Barley (*Hordeum vulgare*)	Higher concentration caused chlorosis and necrosis; suppressed mineral uptake	Rahman et al. (2005)
Cd	17–72 mg kg^{-1}	Hemp (*Cannabis sativa*)	Reduced growth and photosynthesis	Linger et al. (2005)
Pb, Cd, Cu, Hg	0.1–2.5 mM	Bean (*Phaseolus vulgaris*)	Decline in total chlorophylls	Zengin and Munzuroglu (2005)
Ag, Zn, Pb	10–50 mM	Mung beans (*Vigna radiate*)	Inhibited germination	Ashraf and Ali (2007)
Cd	150 μM	Almond (*Prunus dulcis*)	Decrease in chlorophylls; oxidative damage	Nada et al. (2007)
Cd	0.3, 0.6 and 0.9 mM	Maize (*Zea mays*)	Oxidative damage; loss of chlorophyll	Ekmekçi et al. (2008)
Cd, Cu	Variable	Pea (*Pisum sativum*)	Decrease in root and shoot length, chlorophyll pigments	Hattab et al. (2009)
Cd	100–500 μM	Potato (*Solanum tuberosum*)	Depression in growth and biomass	Gonçalves et al. (2009)
As	—	Rice (*Oryza sativa*)	Reduced percent germination and growth	Shri et al. (2009)
Cd	10–50 μM	Wheat (*Triticum aestivum*)	Suppressed growth and chlorophyll contents	Ci et al. (2010)
Cd, Co, Pb, Ag	50 and 100 μM	*Aeluropus littoralis*	Decrease in photosynthetic pigments	Rastgoo and Alemzadeh (2011)
Fe, Pb, Cu	0.001–1%	Tomato (*Solanum lycopersicum*)	Inhibition in germination, decreased root and shoot length, and biomass	Yaqvob et al. (2011)
Zn, Cd, Cr	Varying	Barley (*Hordeum vulgare*)	Growth abnormalities and affected chlorophylls	González et al. (2012)

(continued)

Table 1 (continued)

Heavy metals	Concentration	Target crop plants	Effect	References
Cd	1, 10 and 50 µM	Lettuce (*Lactuca sativa*)	Decrease in plant growth and photosynthesis	Dias et al. (2013)
Cd	50.0 mM	Chickpea (*Cicer arietinum*)	Abnormal germination and seedling growth	Mondal et al. (2013)
Co, Cd, Pb	500–1250 ppm	Pea (*Pisum sativum*)	Reduced growth and biomass	Majeed et al. (2019)

reduce water content which collectively contribute to germination inhibition of the exposed seeds (Nagajyoti et al. 2010). Schutzendubel and Polle (2002) asserted that heavy metals exert their toxic effects at the cellular level and at plasma membrane which negatively affect cellular activities.

2.2 *Effect on Nutrient Uptake*

After seed germination occurs, protrusion of radicle actively participates in absorbing nutrients and water for carrying out metabolic activities which are required for growth and development of tissues. The presence of heavy metals in the medium or soil may cause injury to radicle/roots which disturbs the whole process of nutrient and water absorption. Toxic metal ions and several other factors operating in soils stimulated by heavy metal result in poor root hair development, root injury, and root death that cause impairment in nutrient and water absorption by plants (Rucińska-Sobkowiak 2016). In wheat seedlings exposed to Cd stress, a significant decline in the uptake of nitrate and potassium was reported by Veselov et al. (2003). Water-deficit conditions along with physiological abnormalities were observed in sunflower grown under Pb, Cd, Cu, and Zn stress (Kastori et al. 2008). Impaired mobilization of nutrient accompanied by suppressed enzyme activity in beans was caused by Cd stress (Sfaxi-Bousbih et al. 2010). Lamhamdi et al. (2013) recorded that spinach and wheat plants exposed to Pb toxicity exhibited lesser nutrient absorption than non-treated plants. In a recent study, Vezza et al. (2018) confirmed that arsenic stress caused a significant decline in water absorption of soybean.

2.3 *Heavy Metals and Oxidative Stress: Impairment in Physicochemical Activities*

Oxidative stress is a common feature of plants when they are challenged with stressful conditions. During stresses, plants produce reactive oxygen species (ROS) and free radicals as by-product molecules which hinder the activities of several enzymes and cause damage to cellular components (Kovtun et al. 2000). An important characteristic of heavy metals is their role in triggering oxidative stress by producing ROS such as hydroxyls (OH−) and superoxide (O−) which then cause a number of abnormalities including enzyme deactivation and tissue damage (Fryzova et al. 2017). Most of the heavy metals have also the tendency to bind with oxygen and many other atoms, which cause inactivation of specific enzymes (Schutzendubel and Polle 2002). In addition, the accumulation of heavy metal causes disturbance in cellular homeostasis (Yadav 2010). The collective result of oxidative stress, imbalanced homeostasis, and binding of heavy metals with other molecules is the impaired physiological and biochemical processes of plants.

In previous findings, reduced enzymatic activity (Rubisco, superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase, and catalase) and protein oxidative damage in barley were observed when the plants were treated with excessive Cu (Demirevska-Kepova et al. 2004). Linger et al. (2005) documented reduced photosynthesis and chlorophyll synthesis in cannabis grown under heavy Cd stress. Xiong et al. (2006) demonstrated the toxic effect of Cu on Chinese cabbage which was evident in reduced activity of nitrate reductase, chlorophyll pigments, and nitrogen metabolism. Anuradha and Rao (2009) outlined that Cd stress suppressed photosynthesis rate, chlorophylls, and different enzymatic activities in radish plants. Jain et al. (2010) revealed that Zn in excessive quantities caused abnormalities in cell division, photosynthesis, and enzyme function in sugarcane. Ali et al. (2011) recorded reduced photosynthesis, transpiration, and pigment concentration in two barley genotypes when they were provided with Al and Cr heavy metals. Ghani (2011) observed a decline in chlorophyll contents and mineral uptake by *Brassica juncea* in response to Cr toxicity. Shu et al. (2012) documented that Pb at higher concentration negatively affected chlorophyll, carotenoids, and superoxide dismutase *Jatropha curcas*. Dias et al. (2013) reported the similar toxic effect of Cd on photosynthesis, Rubisco, and CO_2 assimilation in lettuce. Sheetal et al. (2016) reported that different heavy metals (Cd, Pb, Hg, Cr) adversely affected photosynthesis and chlorophyll contents resulting in reduced biomass of mustard.

Plants exposed to biotic or abiotic stress often compensate for the adverse effect of stress by mobilizing resources, activating enzymes, and promoting programmed cell death and several other mechanisms. To counteract the oxidative stress induced by heavy metals, the antioxidant system of plants (comprising enzymes and molecules) recognizes signaling molecules and responds accordingly by adjusting the expression of genes, and metabolic physiology (Foyer and Noctor 2005). Thus, oxidative stress induced by heavy metals is itself a regulatory mechanism to overcome the drastic consequences of metal toxicity.

2.4 Growth and Yield Responses to Heavy Metal Stress

Growth and yield of crop plants are linearly related to the proper functioning of cellular machinery, roots, absorption capacity of minerals and nutrients, photosynthesis, transpiration, and efficient working of different enzymes. Malfunctioning in these processes can lead to retarded growth and yield of crops. As it is evident that heavy metals cause cell membrane disruption (Llamas et al. 2008), abnormalities in antioxidative enzymes (Zhang et al. 2007), photosynthesis and pigments (Sheetal et al. 2016), respiration (Lösch 2004), transpiration and stomatal conductance (Haag-Kerwer et al. 1999; Rucińska-Sobkowiak 2016), and mineral and water absorption (Ayangbenro and Babalola 2017), these abnormalities lead to reduced growth, biomass, and yield of crop plants which are challenged with heavy metal stress. In earlier investigations, reduced growth, biomass, and yield in wheat (Shukla et al. 2003), cotton (Wu et al. 2004), wheat (Singh et al. 2007), sugar beet (Singh et al. 2008), *Vigna radiate* (Singh and Agrawal 2010), lady's finger (Sharma et al. 2010), brinjal (Gond et al. 2013), mustard (Sheetal et al. 2016), and sorghum (Sihag and Joshi 2018) have been reported.

3 Management Approaches for Heavy Metal Pollution

Soils polluted with heavy metals pose a challenging task to soil biologists since heavy metals not only affect plant growth but also negatively modify the soil microorganisms. Besides their drastic effects on plants and microbes, heavy metals may make their way to the entire food chain resulting in severe implication on human health. Wuana and Okieimen (2011) have presented a comprehensive review about remediation methods of soils where heavy metals prevail. They described that major methods which address the reclamation of heavy metal-polluted soils are immobilization techniques, solidification, soil washing, verification, and phytoremediation. However, they argued that employment of a particular method as a remediating tool strongly depends on the physical and chemical characteristics of soils. Yao et al. (2012) highlighted three broad approaches which include physical, chemical, and biological techniques to reclaim heavy metal-polluted soils. Ali et al. (2013) have weighed the utilization of phytoremediation techniques in polluted soils. They highlighted the significance of different plants as phyto-extractants of heavy metals. Gupta et al. (2015) suggested that biosorption—which employs the use of biosorbents, e.g., microbes, biological wastes, and cellulose—is an effective technique to remove heavy metals from polluted soils with costs affectivity. Majeed et al. (2019) reviewed the possibilities of plant growth-promoting bacteria in alleviating the adverse effects of contaminated soils on plants and as potential remediating tools in agriculture.

4 Conclusions

Heavy metals are significant sources of soil pollution which originates from natural weathering processes as well as from extensive anthropogenic actions. Industries, mining, wastewater disposal, and use of agrochemicals in agriculture are leading sources of heavy metals in soils. The occurrence of heavy metals has drastic consequences on soil microorganisms, animals, and plants; thus, they disturb the whole ecosystem. Plants and cultivated crops require healthy and pollution-free soils for germination, establishment, and proper growth. The presence of heavy metals in soil definitely imparts negative effects on metabolic, physiological, and developmental aspects of crop plants which result in reduced production and final yields. The metallic stress generally creates ROS, and oxidative stress at the cellular and tissue levels of crop plants. The stress conditions adversely affect photosynthesis, respiration, transpiration, water and nutrient absorption, nutrient mobilization, and accumulation inside tissues. Although different crop plants vary in their tolerance to heavy metal stress, literature reviews suggest that at higher concentrations nearly all the heavy metals are toxic and almost all plants are susceptible to the adverse consequences of their toxicity. Heavy metal-polluted soils are reclaimed by physical, chemical, or both physical and chemical, and biological, methods.

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Heavy Metal-Induced Toxicity Responses in Plants: An Overview from Physicochemical to Molecular Level

Ritu Chaturvedi, Lovey Talwar, Garima Malik, and Manoj S. Paul

Contents

1 Introduction

Extensive industrialization coupled with unsustainable development approach has generated wastes and pollutants that have long-term detrimental effects on both terrestrial and aquatic ecosystems. In the name of development, reckless anthropogenic activities have exposed our environment to a range of organic and inorganic pollutants. Out of these, the intractable and persistent nature of heavy metals (HMs) along with their tendency to bioaccumulate makes them a pollutant of worldwide concern. HMs are loosely defined group of elements having atomic mass >20

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(excluding alkali metals) and specific gravity >5, exhibiting metallic properties (Rascio and Navari-Izzo 2011). Out of the 118 known chemical elements, 91 are metals, of which 53 are HMs. Some HMs such as Zn, Cu, Ni, Mn, Co, and Mo serve as essential micronutrients and are required for vital physiological pathways (Shahid et al. 2015). But others such as As, Pb, Cd, Hg, and Cr have no known biological role and prove to be toxic if their accumulation surpasses optimal concentrations (Pierart et al. 2015). The bioavailability of HMs is limited due to their strong affinity to soil particles and low solubility in water. However, the exudation of carboxylates and acidification of the rhizosphere lead to enhanced HM bioavailability (Clemens et al. 2002). Further, the extent of uptake of HMs by plants is also governed by the concentration of organic and inorganic matter, soil pH, temperature, and redox potential (Benavides et al. 2005).

Since enzymes are the key targets of HMs, their presence in soil can disrupt soil enzyme activity markedly. The toxicity resulting from HM exposure in plants encompasses a range of interactions at cellular level such as protein inactivity or enzyme denaturation (Hall 2002). All plant species modulate mechanisms such as uptake/efflux, transport/sequestration of HM in vacuoles, chelation to phytochelatins/metallothioneins, and actuation of antioxidants that allocate HM tolerance at basal level (Viehweger 2014; Shahid et al. 2015).

Heavy metal toxicity is known to disrupt the redox status of cells and leads to enhanced accumulation of reactive oxygen species (ROS) followed by oxidative damage. ROS comprises both free radical, i.e., superoxide ($O_2^{\bullet-}$), $OH^{\bullet}$, hydroxyl, $HO_2^{\bullet}$, perhydroxy and $RO^{\bullet}$, alkoxy, and molecular (non-radical) forms of O_2. ROS are also produced continuously as a result of various physiological reactions localized in intracellular compartments such as chloroplast, mitochondria, and peroxisomes (Gill and Tuteja 2010). Generally, there exists a balance between ROS production and detoxification by virtue of various antioxidative defense mechanisms. But in conditions of various abiotic or biotic stress factors such as temperature, drought, salinity, HMs, and pathogen attacks, this equilibrium gets disturbed leading to ROS accumulation which causes damage to intracellular machinery.

Apart from the detrimental effects on flora and fauna, the presence of HMs in environment has deleterious impact on soil health by disturbing pH, organic carbon, and cation-exchange capacity (Tiwari and Lata 2018) which further leads to imbalances in ecological systems such as habitat destruction, loss of biodiversity, and poor vegetation development (Prakash et al. 2019).

2 Effect of Toxic HMs on Growth and Physiology of Plants

HMs tend to accumulate and affect physiological and molecular reactions in plants adversely, leading to decline in crop productivity (Tiwari and Lata 2018). The physiological and biochemical effects of HM exposure are under scrutiny due to their tendency to bioaccumulate and enter food chain (Shahid et al. 2014). Several studies have been carried out recently to examine HM toxicity, uptake, sequestration, detoxification, and tolerance at physiological and molecular levels. Excessive accumulation of HMs is known to affect seed germination, plant growth, biosynthesis of chlorophyll, photosynthesis, respiration, and overall metabolism adversely in plants (Singh et al. 2010).

ATSDR (Agency for Toxic Substances and Disease Registry) has ranked As, Pb, Hg, and Cd as first, second, third, and seventh, respectively, in its substance priority list 2017 as the most toxic HMs, based on the frequency of occurrence and severity of toxicity. Arsenic (As) is a naturally occurring metalloid originating via volcanic action, erosion of rocks, and anthropogenic activities such as mining, smelting, and use of pesticides (Neumann et al. 2010). In the environment, As exists in two forms (inorganic arsenate As(V) and arsenite As(III)), both of which are extremely toxic. However, As(III) is considered to be more toxic than As(V) since it interrupts biological functioning, disturbs metabolism, and generates ROS in plants, whereas As(V) interferes with oxidative phosphorylation and ATP synthesis during energy metabolism (Verma et al. 2016).

Lead (Pb) is one of the most widely present trace metals which is evenly distributed in natural sources. Pb occurs in many forms in which Pb^{2+} is extremely toxic to environment due to its nonbiodegradable nature. The use of leaded fuels in transport, plumbing, and painting elements contributes to anthropogenic sources of Pb pollution. Pb hampers basic metabolic processes in plants such as seed germination and development of seedling, elongation of root and cell division, photosynthesis, and transpiration (Pourrut et al. 2011). Pb is highly phytotoxic due to its ability to block active sites of enzymes and replace essential ions leading to changes in cell membrane permeability. Pb stress leads to overproduction of ROS and may inhibit ATP production and induce lipid peroxidation and DNA damage (Pourrut et al. 2011).

Mercury (Hg) is naturally present in earth's crust but its accumulation in natural resources is due to anthropogenic activities (Montero-Palmero et al. 2014). Hg exists in many forms in the environment such as elemental or metallic (Hg^0), organic (CH_3-Hg), inorganic (Hg_2Cl_2), and ionic (Hg^{2+}), of which ionic form is the most prevalent (Zhou et al. 2008). Though it may not cause significant harm at lower concentrations, it is highly phytotoxic if accumulated in higher concentrations. It can hinder water flow in plants by binding with water channel proteins leading to stomatal closure (Zhou et al. 2008). Besides, it has also been reported to induce oxidative stress, disrupt membrane lipids, and interfere with mitochondrial activity (Zhou et al. 2007).

Due to its high solubility in water, Cd is regarded to be the most phytotoxic HM. Since it is a commonly discharged pollutant in agricultural lands, it can be readily taken up and accumulated by plants leading to entry into food chain. It is a potent carcinogen and crop plants have been reported to be the main source of Cd exposure in humans (Gill and Tuteja 2011). Cd is known to hinder activities of several enzymes participating in basic metabolic reactions such as photosynthesis and growth, disrupt antioxidant machinery, and induce oxidative stress (Gill and Tuteja 2011).

3 Generation of ROS

Plants are known to produce increased quantities of reactive oxygen species (ROS) at some stage as a consequence of abiotic/biotic stress exposure. Even though molecular oxygen is fairly nonreactive, its consecutive reduction to water during cellular metabolism yields toxic intermediates which include (a) oxygen-derived free radicals such as hydroxyl (OH^-), superoxide anion ($O_2^{\bullet-}$), peroxyl ($RO_2^\bullet$), and alkoxyl ($RO^\bullet$) radicals or (b) oxygen-derived non-radical species such as hydrogen peroxide (H_2O_2), organic hydroperoxide (ROOH), and singlet oxygen ($^1O_2$) (Scandalios 2005; Shahid et al. 2014). Further, the presence of transition metals (such as Cu, Cr, and Fe) enables Haber-Weiss mechanism or Fenton reaction to yield $OH^\bullet$, considered to be the most reactive species biochemically (Gill and Tuteja 2010). Figure 1 depicts ROS generation from molecular oxygen.

Triplet oxygen ($^3O_2$) or dioxygen or molecular oxygen is in the electronic ground state and hence most stable and common allotrope of oxygen. Out of the total O_2 consumed by plants, around 1–2% is digressed to generate ROS in various organelles (Bhattachrjee 2005). As shown in Fig. 1, O_2 upon reduction yields $O_2^{\bullet-}$ and O_2^{2-}, which cannot pass through biological membranes and readily dismutate at low pH to yield H_2O_2. Singlet oxygen ($^1O_2$) is the first excited electronic state of $^3O_2$, formed by the reaction between photoexcited (triplet) state of chlorophyll and $^3O_2$. Its formation is also favored during conditions of abiotic stresses when the intracellular concentration of CO_2 is low due to stomatal closure. Due to its very reactive nature, it possesses very serious damaging effect on photosynthetic machinery including photosystem (PS) I and II. (Gill and Tuteja 2010). Superoxide radicals ($O_2^{\bullet-}$) are formed perpetually during photosynthesis as a result of partial reduction of O_2 during noncyclic pathway in thylakoid membrane. Their formation is also inevitable during aerobic respiration wherein O_2 may react with the components of electron transport chain (ETC) to yield $O_2^{\bullet-}$. Though $O_2^{\bullet-}$ is moderately reactive, short lived (half-life: 2–4 μs), and usually the first ROS to be generated, they can trigger the formation of more reactive ROS as shown in Fig. 1.

The univalent reduction of $O_2^{\bullet-}$ yields H_2O_2 which is also moderately reactive but possesses a relatively longer half-life (1 ms). It is a potent inducer of oxidative stress in plants and is capable of inactivating enzymes by oxidizing thiol groups. Though at low concentrations H_2O_2 acts as a signaling molecule during stress and is being

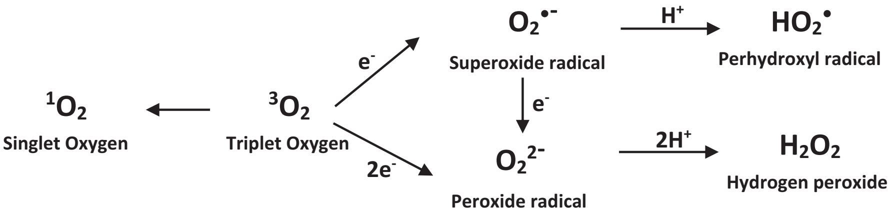

Fig. 1 ROS generation from molecular oxygen

regarded as second messenger due to its relatively longer half-life and permeability across membranes (Quan et al. 2008), it can trigger programmed cell death at high concentrations. Hydroxyl radicals ($OH^{\bullet}$) are one of the most reactive ROS known. As shown in Fig. 2, transition metals can lead to the generation of $OH^{\bullet}$ from $O_2^{\bullet -}$ and H_2O_2 via Fenton reaction. Overproduction of $OH^{\bullet}$ can induce cell death since it is potentially capable of reacting with all biological molecules and cellular machinery leading to oxygen toxicity.

HMs lacking redox capacity (Pb^{2+},Cd^{2+}, Hg^{2+}) are able to enhance the prooxidant status by reducing glutathione pool, activate Ca^{2+}-dependent systems, and affect Fe-mediated processes (Pinto et al. 2003). They can also lead to the production of by $O^{2\bullet -}$ and $^1O_2$ by disrupting the photosynthetic electron chain. ROS possess unpaired electrons in valence shell and are unstable and short lived but very reactive molecules chemically (Wang et al. 2010). The equilibrium between steady-state levels of ROS is regulated by the reciprocity between ROS production and detoxification mechanisms, which is ultimately guided by the physiological, biochemical, developmental, and environmental stimuli (Benavides et al. 2005). A pictorial representation of different ROS-generating and -detoxifying mechanisms has been illustrated in Fig. 2.

4 Sites of ROS Production in Plants

Green plants are particularly at the peril of oxidative damage due to oxygenic conditions and composition of chloroplast envelope (Gill and Tuteja 2010). ROS production is the outcome of interactions between HMs and ETC (electron transport chain), operating in chloroplast and mitochondrial membranes. Chloroplast and peroxisomes are the main sites of ROS generation under light conditions, whereas mitochondria are the main organelle involved during dark conditions. Besides these, ROS are also generated in cytoplasm and endoplasmic reticulum during detoxification reactions involving cytochrome P450. Cell wall peroxidases, germin-like oxalate oxidases, and polyamine oxidases are all sources of H_2O_2 in apoplasts. ROS are also generated in plasma membrane by virtue of NADPH-dependent oxidases. The NADPH oxidase generates $O_2^{\bullet -}$ by transferring electrons from cytosolic NADPH to O_2, which then dismutates to H_2O_2 (Das and Roychoudhury 2014).

Chloroplast consists of well-regulated thylakoid membranes which sheathes light harvesting machinery and encompasses anatomy for optimal light harvesting (Pfannschmidt 2003). During photosynthesis, O_2 generated can readily accept electrons passing through PSI and PSII (via ETC) to yield $O_2^{\bullet -}$. PSII also accounts for generation of $^1O_2$ when the ETC is over-reduced (Asada 2006). Moreover, the reaction between photoexcited/triplet state of chlorophyll ($^3chl^*$) and $^3O_2$ also generates $^1O_2$ in PSII (Karuppanapandian et al. 2011). Abiotic stress conditions leading to overloading of ETC also generate $O_2^{\bullet -}$ via Mehler reaction (Das and Roychoudhury 2014). Research has shown that even under low-light conditions, $^1O_2$ is a natural by-product of photosynthesis mainly formed at PSII (Buchert and Forreiter 2010).

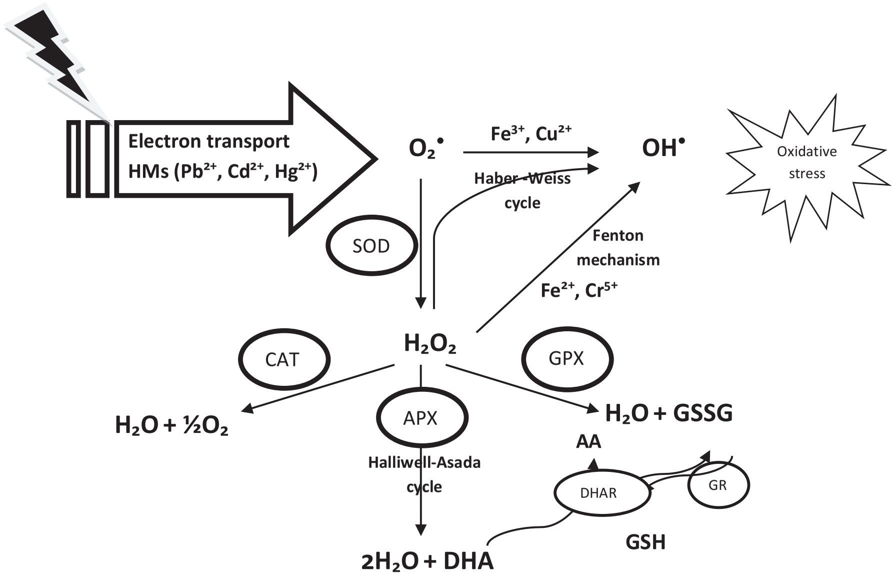

Fig. 2 ROS generation and detoxification by antioxidant enzymes (adapted and modified from Pinto et al. (2003))

Subsequently, on the stromal surface, a membrane-bound Cu/ZnSOD keeps on converting $O_2^{\bullet -}$ into H_2O_2 (Miller et al. 2010) and more toxic ROS like $OH^{\bullet}$ via H_2O_2 intermediate by the Fenton reaction at the Fe-S centers. Though chloroplast is the major source of ROS generation in plant cells, $^1O_2$ accumulating in it can lead to protein damage and peroxidation of its integral lipids and fatty acids, ultimately leading to cell death.

Mitochondria or the powerhouses are also potential sites of ROS generation such as H_2O_2 and $O_2^{\bullet -}$. Presence of specific ETC components, role in photorespiration, and an environment rich in O_2 and carbohydrates (due to photosynthesis) are key features that make plant mitochondria distinct from their animal counterparts (Noctor et al. 2007). The mitochondrial ETC (complexes I and III) abodes electrons with ample free energy and potential to reduce O_2 directly to $O_2^{\bullet -}$, which can be further dismutated to H_2O_2 by SOD. Around 1–5% of O_2 consumed is involved in H_2O_2 production in isolated mitochondria (Moller 2001). H_2O_2 upon reaction with reduced Fe^{2+} and Cu^+ can lead to production of highly toxic $OH^{\bullet}$, which is capable of penetrating membranes and leaving the mitochondrion (Rhoads et al. 2006). A common outcome of $OH^{\bullet}$ generation is lipid peroxidation leading to formation of cytotoxic products capable of reacting with proteins, lipids, and nucleic acids, and ultimately causing cellular damage. ROS generation by mitochondrion is an unavoidable adjunct to aerobic respiration under normal conditions, which gets accelerated due to over-reduction of electron carriers during conditions of stress (Pastore et al. 2007). To combat oxidative stress and control ROS generation, plant mitochondria may employ energy-dissipating systems. Further, mitochondria are also equipped with pivotal enzymes, namely mitochondrial alternative oxidase (AOX) and mitochondrial SOD (Mn-SOD), which help in trimming down ROS generation (Das and Roychoudhury 2014).

Peroxisomes are single lipid bilayer membrane-bound subcellular organelles, possessing oxidative metabolism. Peroxisomes produce $O_2^{\bullet -}$ as a part of their routine metabolism, similar to mitochondria and chloroplasts. $O_2^{\bullet -}$ is generated in the peroxisomal membrane ETC as well as in the matrix. Several metabolic reactions, namely β-oxidation of fatty acids, photorespiratory glycolate oxidase reaction, flavin oxidase pathway, and disproportionation of $O_2^{\bullet -}$ radicals, are responsible for the generation of H_2O_2 in peroxisomes (Gill and Tuteja 2010). Under conditions of high temperature or low water availability, the concentration of CO_2:O_2 reduces considerably and causes increased photorespiration. This leads to formation of glycolate, which is oxidized by glycolate oxidase in peroxisomes, releasing H_2O_2 ultimately and making peroxisomes the leading producer of H_2O_2 during photorespiration (Noctor et al. 2002). Though overproduction of ROS leads to oxidative damage and cell death in plants, some research also shows that small concentrations of $O_2^{\bullet -}$ and H_2O_2 are engaged as signaling molecules in plants (McDowell and Dangl 2000). Hence peroxisomes can regarded as organelles capable of contributing to a better consolidated communication system among cellular compartments by generating and releasing vital signaling molecules such as H_2O_2, $O_2^{\bullet -}$, and $NO^{\bullet}$ into the cytosol (Corpas et al. 2001).

5 Targets of ROS Generated in Plants

ROS generation is known to damage vital biomolecules, namely lipids, proteins, and nucleic acids, which hampers cellular functioning, ultimately leading to cell death.

Lipids are the integral part of plasma membrane and play a vital role in cellular integrity and metabolism. Lipid peroxidation is a single, sufficient parameter tested to estimate the amount of membrane damage occurring due to stress. ROS upon crossing a certain threshold level lead to peroxidation of lipids and formation of cytotoxic products capable of exacerbating cellular damage. The ester linkage between glycerol and fatty acids and the double bond between C atoms (C=C) are the two main sites prone to ROS attack in membrane phospholipids. Further, $OH^•$ can trigger a cyclic chain reaction to peroxidate the polyunsaturated fatty acids present in membranes leading to membrane damage. Lipid peroxidation affects membrane fluidity, renders the membrane leaky to molecules which would have otherwise been unable to cross it except by using specific transporters, and causes damage to the membrane proteins, disband membrane receptors, ion channels, and membrane-localized enzymes (Gill and Tuteja 2010).

Proteins are prone to reversible or irreversible covalent modifications induced by ROS (Ghezzi and Bonetto 2003). ROS, irrespective of their location of generation, probably target proteins which in turn respond with different susceptibilities based on their composition. Proteins composed of amino acids like lysine, arginine, proline, threonine, and tryptophan are susceptible to site-specific modification and proteolytic degradation (Møller et al. 2007). Proteins composed of thiol groups and sulfur-containing amino acids (cysteine and methionine) are most vulnerable since they fairly reactive $^1O_2$ and $OH^•$. Proteins containing Fe-S centers upon oxidation with $O_2^{•-}$ get irreversibly inactivated. Proteins can undergo direct or indirect modifications; direct modification involves a chemical modification (carboxylation, disulfide bond formation, nitrosylation) to alter protein activity, whereas damage upon reaction with products of lipid peroxidation in oxygenic conditions is an indirect modification.

DNA: Chloroplast and mitochondrial DNA are more prone to oxidative damage than plant nuclear DNA, due to the proximity to ROS generation machinery. ROS can initiate endogenous or spontaneous DNA damage in many ways including base deletion, formation of pyrimidine dimers, strand breaks, cross-links, and modification of bases by alkylation and oxidation (Gill and Tuteja 2010). Different nucleotide bases respond differently to ROS; for example guanine is predominantly attacked by $^1O_2$ whereas not at all by $O_2^{•-}$ and H_2O_2. $OH^•$ is highly reactive and can damage all four nucleotide bases along with the deoxyribose backbone. It can also react with DNA or associated proteins to create DNA-protein cross-links which cannot be repaired easily and prove to be lethal for the plant cells. DNA damage can result in errors during replication, arrest or induction of transcription, and reduction in protein synthesis and signal transduction pathways leading to genomic instability besides affecting overall growth and development.

6 HM Tolerance Mechanisms in Plants

All plants presumptively exhibit elementary HM tolerance by regulating a nexus of uptake/efflux, transport/sequester, and chelation (Viehweger 2014). These pivotal elements play the decisive role in determining the hyperaccumulating, hypertolerating, or non-accumulating nature of plants. While hyperaccumulating plants are able to translocate and accumulate high concentration of HMs in aerial parts without suffering phytotoxicity, hypertolerant plants are capable of excluding HMs accumulating, especially in aerial parts. Since efflux and sequestration are the key elements leading to basal tolerance (Clemens 2001), they do happen in specific plant structures, namely cuticle, epidermis, and trichomes (Shahid et al. 2014), where they may cause damage to photosynthetic machinery, if not detoxified. In order to cope up with stress, plants are equipped with mechanisms at every level. While some of these mechanisms may either altogether prohibit the entry of HMs into plants or increase the excretion of HMs by roots, others may lead to binding of HMs to the cell wall, or chelation of HMs by organic molecules followed by sequestration in vacuoles (Tang et al. 2010).

6.1 Primary-Level Mechanisms of HM Tolerance

HMs primarily gain entry into plants through roots. At entry level, the cell wall and plasma membrane are the first structures that encounter HM stress. Immobilization of HMs by the root cell wall and extracellular carbohydrates serves as the first barrier against HM toxicity. The thickness of roots may increase in order to adsorb HMs onto the surface and reduce its absorption as a response to HM toxicity. Further the selective permeability of plasma membrane excludes many HMs from gaining entry into the cytosol. However, the efficiency of these structures is governed by the intensity of exposure along with species involved. In order to restrict the translocation of HMs absorbed by roots to aerial parts, HMs are either detoxified (complexed with organic acids or amino acids) or sequestered into vacuoles (Shahid et al. 2014). Increased sequestration of HMs in root cells can be achieved by precipitation of HMs as insoluble salts in intercellular spaces, accumulation in plasma membranes, immobilization of HMs by negatively charged pectins within the cell wall, or sequestration in the vacuoles of rhizodermal and cortical cells (Shahid et al. 2014).

6.2 Secondary-Level Mechanisms of HM Tolerance

Plants exhibit homeostatic cellular mechanisms in order to minimize the possible damage caused due to HM exposure. After absorption of HMs, toxicity can be evaded by plants if they possess efficient sinks to store HMs. Vacuoles are such multifunctional organelles that function for metal homeostasis and detoxification by sequestering HMs. This takes place either as a result of ligand binding or by vacuolar entrapment using transporters. Several families of transporters involved in HM homeostasis have been identified using genome sequencing in plants, namely heavy metal ATPases (HMAs), ATP-binding cassettes (ABC), Zrt/Irt-like protein (ZIP), natural resistance-associated macrophage (NRAMP), cation exchangers (CAXs), and cation diffusion facilitators (CDF). Of these, ABC, CDF, and NRAMP have been identified as being crucial for HM tolerance (Chaffai and Koyama 2011).

Metallothioneins (MTs) and phytochelatins (PCs) are crucial and the best characterized HM-binding ligands in plants. The responsiveness of plants to HMs is determined by an allied system of physiological and molecular mechanisms comprising uptake and acquisition of HMs via binding and chelation to polypeptides, namely MTs and PCs; induction of defense metabolites; and alteration of plant metabolic pathways to provide rapid defense and repair (Benavides et al. 2005). HM accumulation in plants is generally a function of uptake capacity and intracellular binding sites. The concentration and affinity of phytochelatins along with the presence and specificity of transporters govern the uptake kinetics (Clemens et al. 2002).

Chelation of HMs by ligand has been a regular mechanism for HM detoxification in organisms, which can be followed by subsequent compartmentalization of ligand-HM complex in vacuoles to prevent free circulation of ions in cytosol. MTs are small gene-encoded, cysteine-rich polypeptides which are classified on the basis of arrangement of cys residues (Cobbett and Goldsbrough 2002). Class I MTs are widespread in vertebrates whereas class II MTs are found in invertebrates, fungi, and plants. PCs have been confusingly described as class III MTs in this system of classification. PCs are small, enzymatically synthesized cysteine-rich peptides with the structure (g-glu-cys)n-gly, (g-glu-cys)n-b-ala, (g-glu-cys)n-ser, (g-glu-cys)n-glu, (g-glu-cys)n-gln, or (g-glu-cys)n, where n varies from 2 to 11. The biosynthesis of PCs requires glutathione (γ-Glu-CysGly) as substrate and phytochelatin synthase (PCS) (EC 2.3.2.15) as enzyme. PCS is a constitutive enzyme that gets activated only in the presence of HMs post-translationally (Cobbett 2000). Cd along with PCs has been shown to accumulate in vacuoles via ABC transporters (Hall 2002). HM tolerance has also been attributed to extracellular chelation via organic acids, namely malate and citrate.

6.3 HM Transport and Signaling in Plants

The advancement in molecular techniques has led to the identification of several cation transporters in recent years, which are able to transport different HMs across biological membranes. Of these, ZIP and Nramp are the major families of transporters involved in micronutrient uptake (Williams et al. 2000). It is unlikely that specific transporters for HMs occur in organisms and hence HMs are likely to enter cells via transporters with broad specificity (Clemens 2001). Cation transporters that show affinity for both Zn and Cd have also been identified suggesting that inessential HMs are taken up along with essential micronutrients. *Arabidopsis halleri* is known to hyperaccumulate both Zn and Cd (Bert et al. 2003). Further, Cd transport has also been shown by AtNramp3 which is involved in Fe transport in *Arabidopsis thaliana* (Thomine et al. 2000). Transcriptomic studies have shown that at least 30 candidate genes are overexpressed in hyperaccumulator *A. halleri* than non-accumulator *A. thaliana.*

Application of various proteomics techniques such as MALDI-TOF and LC-MS have enabled identification of target proteins that participate in HM detoxification in several plants (Tiwari and Lata 2018). Likewise, several amino acids, organic acids, and secondary metabolites (phenols, α-tocopherol) have been traced to play major roles in HM detoxification (Singh et al. 2016). Receptors/ion channels percept HM stress and along with nonprotein messengers (Ca^{2+}, H^+, cyclic nucleotides) they initiate stress signal transduction. These stress signals are relayed by various kinases and phosphatases leading to gene expression of transcription factors (TFs) synthesizing metal-detoxifying peptides (Kumar and Trivedi 2016). Distinct signaling pathways, namely mitogen-activated protein kinase (MAPK), ROS signaling, hormone signaling, and calcium-dependent signaling, are activated by HMs and enhance the expression of stress-responsive genes (Kumar and Trivedi 2016). Numerous TFs can be phosphorylated by MAPK signaling cascade as a response to HM stress. Likewise, alterations in cytosolic Ca^{2+} concentrations are sensed by numerous Ca^{2+} sensors like Ca^{2+}-dependent protein kinases (CDPKs), calmodulins (CaMs), CaM-like proteins, and calcineurin B-like proteins (CBLs) and conveyed to induce stress response (Steinhorst and Kudla 2014). Phytohormone signaling pathways like auxin, ethylene, and jasmonic acid (JA) are also key mechanisms to counter HM stress as variation in the levels of phytohormones affects plant response to HM stress. Exposure to phytohormones can improve antioxidant response in plants during HM stress (Singh and Shah 2014).

6.4 ROS-Induced Defense Responses in Plants

ROS overproduction can distort the redox status of plant cells resulting in oxidative damage that leads to degeneration of biomolecules, dismantling of membranes, lipid peroxidation, ion leakage, and DNA strand cleavage (Shahid et al. 2014). In order to combat oxidative damage occurring during stress conditions, plants have evolved an array of defense mechanisms to transform ROS into less toxic products. These mechanisms help plants to sustain their cellular redox state and mitigate the damage caused by oxidative stress. Majority of these mechanisms rely on synthesis of metabolic intermediaries comprising two arms: (1) nonenzymatic and (2) enzymatic components. Records of HM-induced increase in nonenzymatic and enzymatic antioxidants have been summarized in Table 1.

Table 1 Upregulation of enzymatic and nonenzymatic antioxidants upon exposure to HMs in plants

Antioxidant	HM	Plant species	Reference
Nonenzymatic			
Tocopherol (Vit. E)	Cu	*Anabaena doliolum*	Srivastava et al. (2005)
Ascorbic acid (Vit. C)	Cd, Hg	*Hordeum vulgare, Medicago sativa*	Demirevska-Kepova et al. (2006), Zhou et al. (2007)
Glutathione	Cd, Hg	*Pisum sativum, Sedum alfredii, Vigna mungo, Medicago sativa*	Metwally et al. (2005), Sun et al. (2007), Molina et al. (2008), Zhou et al. (2007)
Phenolics	Cd, Zn	*Kandelia obovata*	Chen et al. (2019)
Carotenoids	Pb	*Arabidopsis thaliana*	Baek et al. (2012)
Proline	Cd, Ni	Microalga (*Chlamydomonas reinhardtii*), *Pisum sativum*	Siripornadulsil et al. (2002), Gajewska and Skłodowska (2005)
Enzymatic			
CAT	Cd	*Oryza sativa, Brassica juncea, Triticum aestivum, Cicer arietinum, and Vigna mungo*	Hsu and Kao (2004), Mobin and Khan (2007), Khan et al. (2007), Hasan et al. (2008), Singh et al. (2008)
	Pb	*Eichhornia crassipes, Acalypha indica*	Malar et al. (2014), Venkatachalam et al. (2017)
	Hg	*Sesbania grandiflora*	Malar et al. (2015)
SOD	Pb	*Eichhornia crassipes, Acalypha indica*	Malar et al. (2014), Venkatachalam et al. (2017)
	Cd	*Hordeum vulgare, Arabidopsis thaliana, Oryza sativa, Brassica juncea, Triticum aestivum, Cicer arietinum, Vigna mungo, Hibiscus cannabinus*	Guo et al. (2004), Skorzynska-Polit et al. (2003), Hsu and Kao (2004), Mobin and Khan (2007), Khan et al. (2007), Hasan et al. (2008), Singh et al. (2008), Feng-tao et al. (2013)
	Hg	*Sesbania grandiflora*	Malar et al. (2015)
APX	Pb	*Eichhornia crassipes, Acalypha indica*	Malar et al. (2014), Venkatachalam et al. (2017)
	Cd	*Brassica juncea, Triticum aestivum, Vigna mungo, Ceratophyllum demersum, Hibiscus cannabinus*	Mobin and Khan (2007), Khan et al. (2007), Singh et al. (2008), Arvind and Prasad (2003), Feng-tao et al. (2013)
	Hg	*Sesbania grandiflora*	Malar et al. (2015)
POX	Hg	*Sesbania grandiflora*	Malar et al. (2015)
GPOX	Cd	*Arabidopsis thaliana, Triticum aestivum, Ceratophyllum demersum*	Skorzynska-Polit et al. (2003), Khan et al. (2007), Arvind and Prasad (2003)
GR	Cd	*Capsicum annuum, Arabidopsis thaliana, Vigna mungo, Triticum aestivum, Brassica juncea*	Leon et al. (2002), Skorzynska-Polit et al. (2003), Singh et al. (2008), Khan et al. (2007), Mobin and Khan (2007)

6.5 Nonenzymatic Components

These include various groups of bioactive molecules, namely tocopherols, ascorbic acid (AA), reduced glutathione (GSH), phenolics, carotenoids, proline, etc. Besides protecting cellular components from damage, they play key roles in plant growth and development (de Pinto and De Gara 2004).

Tocopherols and tocotrienols, together known as tocochromanols, are lipophilic antioxidants belonging to group of vitamin E, known to scavenge ROS and lipid radicals (Falk and Munné-Bosch 2010). Out of the four isomers (α-, β-, γ-, δ-) of tocopherols identified in plants on the basis of the number and position of chromanol ring system, α-tocopherol possesses the highest antioxidant activity as it consists of three methyl groups. Tocopherols can only be synthesized by photosynthetic plants and hence localized in green tissues only. Since chloroplasts of higher plants contain significant amount of α-tocopherol, they are secure against photooxidation since they can react with O_2 and quench its excess energy (Das and Roychoudhury 2014). Tocopherols are also known to protect thylakoid membranes against lipid peroxidation similar to carotenoids (Moucheshi et al. 2014). By halting the chain propagation step and reducing the lipid radicals ($RO^•$, $ROO^•$), tocopherols themselves get oxidized as tocopheroxyl radical ($TOH^•$) which later reacts with GSH and AA to get recycled into its reduced form (Igamberdiev et al. 2004).

AA (vitamin C) is the most widely present and studied antioxidant. Because of its tendency to act as a reducing agent in a number of biological reactions, it is considered to be a potent antioxidant. It is water soluble and synthesized by Smirnoff-Wheeler pathway in plant mitochondria. It acts as a first line of defense against ROS because of its substantial presence in cytosol and apoplast (Barnes et al. 2002) in its reduced form (ascorbate) under normal physiological conditions. The regeneration of ascorbate from fully oxidized dehydroascorbic acid is crucial because it has a short half-life and would be bygone unless it is reduced back. AA can directly reduce $O_2^{•-}$, $^1O_2$, $OH^•$, and H_2O_2 and regenerate α-tocopherol from $TOH^•$, in order to protect membranes from oxidative stress.

GSH is a cysteine-containing, low-molecular-weight thiol tripeptide involved in various cellular processes like cell growth, division, differentiation, synthesis, and transport of biomolecules. It is also water soluble like AA and found in almost all cell organelles in its reduced form abundantly. Its elementary role is in thiol-disulfide interactions, where GSH is continuously oxidized to its disulfide form (GSSG) which is recycled back to GSH either de novo or enzymatically in the presence of NADPH-dependent glutathione reductase (GR), ultimately replenishing the cellular GSH pool. GSH is involved in the synthesis of phytochelatins which chelate HMs and aid in detoxification. Both GSH and GSSG play a pivotal role in actuating secondary metabolism, ROS signaling, and antioxidant defense mechanism by regenerating AA via ascorbate-glutathione (ASH-GSH) cycle. The intricate equilibrium between GSH and GSSG significantly conserves the normal redox system of the cell under normal and stress conditions (Moucheshi et al. 2014).

Phenolic antioxidants are of particular importance due to their expression of antioxidant activity in both in vitro and in vivo studies (Trchounian et al. 2016). Out of the five major groups classified (phenolic acids, flavonoids, lignans, tannins, and stilbenes) flavonoids and phenolic acids constitute the widest classes of plant phenolics biosynthesized majorly from phenylalanine, an aromatic amino acid synthesized from shikimic acid pathway. Flavonoids are water-soluble N-deficient plant pigments possessing a three-ring chemical structure (C6-C3-C6). On the basis of their structure, flavonoids can be classified into four classes: anthocyanins (red-purple pigments), flavonols (colorless-pale yellow pigments), flavanols (colorless pigments that become brown upon oxidation), and proanthocyanidins (PAs) or condensed tannins (Petrussa et al. 2013). Flavonoids show varied concentrations in plants depending upon the species, growth stage, and environment conditions. They serve as secondary ROS scavengers and are known to shield photosynthetic apparatus (Das and Roychoudhury 2014). Flavonoids show synergistic amplification in activities of some antioxidants (tocopherol, ascorbate) by interacting with them (Kasote et al. 2015). They also prevent lipid peroxidation by inhibiting enzyme lipoxygenase (Moucheshi et al. 2014).

Carotenoids, the most common tetraterpenoids, are organic lipophilic pigments localized in plastids of plants and other photosynthetic organisms. They are antennae molecules that absorb visible light (450–570 nm) and pass it on to chlorophyll. There are different types of carotenoids in plants but β-carotenes and xanthophylls are the most abundant and commonly studied. Carotenoids serve as antioxidants and protect the photosynthetic machinery in either of four ways: (1) avoiding the formation of $^1O_2$ by quenching $^3chl^*$ (Moucheshi et al. 2014), (2) scavenging $^1O_2$ and generating heat at by-product, (3) involving xanthophyll cycle to dissipate excess excitation energy, and (4) reacting with lipid peroxidation products to terminate the chain reaction (Das and Roychoudhury 2014).

Proline, besides being an osmolyte, is also a potent ROS scavenger and is known to inhibit the damage caused by lipid peroxidation. The accumulation of proline in considerable amounts in plants during stress can be attributed to either increased synthesis or decreased degradation (Verbruggen and Hermans 2008).

6.6 Enzymatic Components

Catalase (CAT; E.C.1.11.1.6) is tetrameric heme-containing enzyme with the potential to dismutate H_2O_2 into H_2O and O_2 directly ($2H_2O_2 \rightarrow O_2 + 2H_2O$). It possesses a very high affinity as well as turnover rate (~six million molecules min^{-1}) for H_2O_2. The unnecessity of reducing agent in reactions catalyzed makes catalases distinctive among other antioxidants. Generally H_2O_2 is generated in peroxisomes due to oxidative stress resulting from β-oxidation of fatty acids, photorespiration, and purine catabolism (Gill and Tuteja 2010). But catalases are also present in cytosol and organelles such as chloroplast and mitochondria (Mhamdi et al. 2010). Several isoforms of *CAT* genes have been reported in higher plants (up to 12 in

Brassica) of which the 3 isoforms in *Zea mays* are found to be differentially localized and independently expressed (i.e., although both *CAT 1* and *CAT 2* are localized in peroxisomes and cytosol *CAT 1* is expressed in pollen and seeds whereas *CAT 2* is expressed in photosynthetic tissues, roots, and seeds; *CAT 3* is localized in mitochondria of leaves and vascular tissues):

$$\mathbf{2H_2O_2 \rightarrow O_2 + 2H_2O}$$

Superoxide dismutase (SOD; E.C.1.15.1.1) is a multimeric metalloprotein and the most effective intracellular antioxidant known to detoxify $O_2^{\bullet-}$ and provide first line of defense against oxidative stress (Gill and Tuteja 2010). Based on the metal cofactor present at the active site, protein folds, and subcellular distribution, SOD isoforms occurring in plants can be characterized as Cu/Zn-SOD (localized in cytosol, peroxisomes, and chloroplasts), Mn-SOD (localized in mitochondria), and Fe-SOD (localized in chloroplasts) (Das and Roychoudhury 2014). SODs catalyze the dismutation of $O_2^{\bullet-}$; that is, one $O_2^{\bullet-}$ is reduced to H_2O_2 and the other $O_2^{\bullet-}$ is oxidized to O_2, henceforth decreasing the risk of Haber-Weiss-catalyzed $OH^{\bullet}$ formation (Gill and Tuteja 2010). Under abiotic stress conditions, the activity of SOD has been found to be upregulated in plants (Boguszewska et al. 2010):

$$\mathbf{O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2}$$

Ascorbate peroxidase (APX; E.C.1.1.11.1) is an intrinsic constituent of ASH-GSH cycle. Using ascorbic acid as a reducing agent, APX transforms H_2O_2 into H_2O and DHA (dehydroascorbate) in water-water and ASH-GSH cycle. While it primarily scavenges H_2O_2 in cytosol and chloroplast, CAT executes the same function in peroxisomes (Das and Roychoudhury 2014). Based on the amino acid composition and subcellular localization, five isoforms originating from alternative splicing (contributing to the differential regulation of expression of various isoforms) have been characterized in plants. Soluble isoforms are found in cytosol (cAPX), mitochondria (mitAPX), and chloroplast stroma (sAPX), while membrane-bound isoforms are found in microbody (including peroxisome and glyoxysome) (mAPX) and chloroplast thylakoids (tAPX) (Caverzan et al. 2012). Since APX possesses a higher affinity for H_2O_2 (μM range) than CAT (mM range) and is widely distributed, it is bound to play a crucial role in H_2O_2 scavenging during stress (Gill and Tuteja 2010):

$$\mathbf{H_2O_2 + AA \rightarrow 2H_2O + DHA}$$

Guaiacol peroxidase (GPOX; E.C.1.11.1.7) is a heme-containing enzyme that scavenges excess H_2O_2 under normal conditions and stress as well. Plant-derived GPX is different from APX both in sequences and its physiological role. In addition to being active in cell wall, GPX is active both intracellularly (cytosol, vacuoles) and extracellularly (Das and Roychoudhury 2014). GPX prefers aromatic compounds (namely guaiacol and pyrogallol) usually as electron donors, oxidizing ascorbate at a rate of around 1% to that of guaiacol (Gill and Tuteja 2010). Besides playing a pivotal role in the biosynthesis of lignin, GPOX also decomposes indole-3-acetic acid (IAA) and provides defense against biotic stresses by consuming H_2O_2:

$$\mathbf{H_2O_2 + GSH \rightarrow H_2O + GSSG}$$

Glutathione reductase (GR; E.C.1.6.4.2) is a flavoprotein oxidoreductase, playing a key role in ROS detoxification by maintaining the reduced status of GSH from GSSG using NADPH as reductant. It is localized mostly in chloroplasts, but also present in mitochondria and cytosol in small amounts. GSH is a compound with low molecular weight that acts as a reducing agent in preventing thiol groups from getting oxidized, and reacts with detrimental ROS members like $^1O_2$ and $OH^{\bullet}$ (Das and Roychoudhury 2014). Since GSH is continuously used up in ASH-GSH cycle to regenerate AA, it gets converted into its oxidized form GSSG. GR is a pivotal enzyme of this cycle as it catalyzes the formation of disulfide bond in GSSG and maintains GSH pool:

$$\mathbf{GSSG + NADPH \rightarrow 2GSH + NADP^+}$$

7 Conclusions and Future Perspectives

The presence of heavy metals in environment is known to exert genotoxic and clastogenic effects on plants. Subsequently plants are equipped with various defense mechanisms which are imperative for their survival. Various omics approaches, namely transcriptomics, proteomics, metabolomics, and ionomics, are being employed to encode regulatory mechanisms involved in HM tolerance in plants. The induction of genes central to HM stress signaling points to a composite cross talk between plant and HM during stress response and tolerance. Therefore, a precise interpretation of the intricate HM stress signaling pathways is of key requirement to elucidate stress response network in plants. Functional genomics techniques can be synergized with omics technologies for the development of improved varieties with enhanced abiotic stress tolerance. This strategy can also be employed to raise genetically engineered plants with enhanced accumulation which can be used not only for phytomining, but also for biofortification.

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Heavy Metal Stress in Plants: Effects on Nutrients and Water Uptake

Saira Siyar, Sajeela Sami, and Abdul Majeed

Contents

1 Introduction

Both wild and cultivated plants encounter several challenges in their life span which impart negative effects on their vital activities. These challenges are biotic as well as abiotic. Heavy metal (HM) pollution on soils is among the drastic abiotic stresses to which plants are confronted with. Sources of heavy metal in soils are natural mineralization of rocks, volcanoes, and industrial and mining activities (Alloway 2012). After the birth of the industrial revolution, an increasing tendency towards operation of diverse processing plants has led to the emission of huge quantities of waste material. Due to the lack of improper handling in most of the waste emissions, they often pollute agricultural soils through irrigation or direct contamination. The industrial waste may contain significant proportions of HM which on reaching to soils could increase the indigenous concentration of HM.

Elevated concentration of HM in soil withering due to natural origins or anthropogenic actions could not be tolerated by most of the plant species. Some HM such as Zn, Fe, and Cu are essentially required in small quantities for plants' nutritional needs while some HM like Cd, Pb, and Hg are nonessential (Göhre and Paszkowski 2006; Khan et al. 2008). Essential HM at above-threshold level while nonessential

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HM at lower concentration is toxic to plants affecting their growth, physiology, and development in several ways. Their interaction with plants could cause oxidative damage and abnormal ionic homeostasis (Yadav 2010). HM stress in soil malfunctions the absorption of nutrients by roots which results in deficiency of nutrients in plants. Siedlecka (1995) asserted that heavy metal stress interacts with other nutrients such as Zn, Cu, Fe, K, Ca, N, and P and disturbs their absorption by roots by either immobilization or decreased uptake. Bertoli et al. (2012) observed reduced micro- and macronutrients in the aerial parts of tomato which was grown in concentrated Cd solution. In many other studies, reduced uptake and distribution of micro- and macronutrients in different plants challenged with heavy metal stress were recorded (Gonçalves et al. 2009; Koleva 2010; Manivasagaperumal et al. 2011; Eker et al. 2013; Gupta et al. 2013; Li et al. 2016).

Drastic consequences of HM toxicity are not restricted to disturbed nutrient uptake by plants. Water status in the soil as well as in plants growing in HM stress often becomes nonoptimal for major growth phenomena. Exceeding concentration of HM in soils and their accumulation by plants result in deficit water status in soil as well as inside plant's tissues. In soil, presence of HM causes osmotic stress (which is augmented in the presence of inorganic salts) and plants under these conditions find it difficult to absorb water properly (Rucińska-Sobkowiak 2016). However greater accumulation of HM inside plants' tissues results in irregular osmosis, transpiration, and stomatal activities (Barceló and Poschenrieder 1990). In this chapter, we discuss the effects of HM stress on plants' nutrient and water status.

2 Role of HM on Nutrient and Water Uptake by Plants

The rhizospheric soil provides an ideal medium for the growing plants. It covers basic requirements, i.e., micro- and macronutrients and water which are necessary for plants' growth and successive developmental processes. Besides acting as a source of nutrients and water, the soil also harbors millions of microbes which drive the ecological dynamics. Availability of balanced amount of nutrients and water in soil depends on several factors among which the chemistry of soil plays a crucial role. Contaminant withering, organic or inorganic, can damage the soil's nutritional, water, and ecological stability.

Soil containing heavy metals above the threshold level adversely affects plants' capacity to absorb water and nutrients properly. Lab studies and field experiments indicate that HM stress often results in poor mineral and water absorption as well as their imbalanced translocation inside tissues although the degree of poor absorption and translocation depend on the type and concentration of HM. In a pot experiment, Liu et al. (2003) demonstrated that excessive Cu concentration altered micro- and macronutrient uptake and their translocation in five rice genotypes. Rahman et al. (2005) recorded a decreased concentration of Mn and Zn in roots and shoots while altered concentration of other micronutrients in barley when plants were subjected to Ni stress. Ahmad et al. (2011a, b) documented that lead (Pb) at a concentration ranging between 0.01 and 1.0 mg L^{-1} significantly reduced K and Cu concentrations in roots, leaves, and shoots of two maize genotypes. In *Brassica napus* and *B. juncea*, suppression of Mn and Zn mineral contents was observed in shoots as a result of Cu stress (Feigl et al. 2015). In several other studies, reduced uptakes of both micro- and macronutrients in different plants exposed to different HM have been observed (Table 1).

Proper availability of water in the soil, its timely absorption by plants, and appropriate translocation in tissues are vitally necessary for several metabolic and physiological events on which the future growth and development depend. Water plays a key role in maintaining the turgidity of plants' cells, channelizing mineral nutrients from soil to roots, and regulating the translocation of food between source and sinks. Thus, balanced water absorption by plants could stimulate a desired

Table 1 Effect of different heavy metals on the nutrient absorption and uptake of different plant species

Plants species	Heavy metal stress	Effect on nutrient uptake	References
Phaseolus vulgaris	Pb	Reduced uptake of Ca, Mn, and Zn	Geebelen et al. (2002)
Oryza sativa	Cd	Negative correlations with Mn^{++} in leaves	Liu et al. (2003)
Nicotiana tabacum	Cd	Fe deficiency	Yoshihara et al. (2006)
Sesuvium portulacastrum and *Mesembryanthemum crystallinum*	Cd	Reduced uptake of K^+ and Ca^{++}	Ghnaya et al. (2007)
Triticum aestivum	As	Disturbed mineral nutrient uptake and translocation	Quanji et al. (2008)
Matricaria chamomilla	Cu and Cd	Reduced uptake of K	Kováčik et al. (2009)
Brassica napus	Zn	Adverse effects on several minerals	Wang et al. (2009a)
Hydrilla verticillata	Zn	Decreased P uptake	Wang et al. (2009b)
Oryza sativa		Decrease in N, P, K, Mn, Cu, Zn, and Fe uptake	Sundaramoorthy et al. (2010)
Vallisneria natans	Pb	Decreased concentration of P, K, and Mn	Wang et al. (2011)
Helianthus annuus	Ni	Reduced uptake of micro- and macronutrients	Ahmad et al. (2011a, b)
Pfaffia glomerata	Hg, As, and Pb	Altered uptake of macro- and micronutrients	Gupta et al. (2013)
Triticum aestivum, Spinacia oleracea	Pb	Reduction in the uptake of Na, K, Ca, P, Mg, Fe, Cu, and Zn	Lamhamdi et al. (2013)
Lactuca sativa	As	Altered uptake of micro- and macronutrients	Gusman et al. (2013)

physiological activity and growth and development. Besides nutrient abnormalities, the role of HM in altered water status and transpiration abnormalities in several plants is well established. Perfus-Barbeoch et al. (2002) summarized the effect of Cd on water relation of *Arabidopsis thaliana*. They asserted that Cd could reduce water contents which influenced guard cells and transpiration flow with negative behavior in stomata. Kastori et al. (2008) correlated HM (Zn, Cu, Pb, and Cd) to reduced water content and transpiration in sunflower. In recent studies it was documented that plant species, i.e., *Cinnamomum camphora*, *Citrus grandis*, and *Kandelia obovata*, exposed to excessive concentrations of Zn, Cu, and Pb experienced abnormalities in stomatal conductance, water uptake, and transpiration (Yang et al. 2018; Li et al. 2019; Shen et al. 2019) (Table 2).

The mechanism of heavy metal-induced deficit water and nutrients is a complex process which may involve (1) metal-mineral complex formation, (2) competition with nutrients for active absorption by roots, (3) osmotic deficit, (4) root injury, (5) accumulation in roots and subsequently in shoots, and (6) xylem blockage (Rucińska-Sobkowiak 2016) (Fig. 1). Metal nutrient interaction depends on the

Table 2 Effect of heavy metal stress on water relation of different plants

Plants species	Heavy metals	Association with water status	References
Helianthus annuus	Zn, Cu, Pb, and Cd	Reduced water content and transpiration	Kastori et al. (1992)
Arabidopsis thaliana, *Vicia faba* and *Commelina communis*	Cd	Water stress and stomatal closure	Perfus-Barbeoch et al. (2002)
Brassica juncea	Cd	Reduced water content	Singh and Tewari (2003)
Vigna radiate	Ni	Reduced water potential and relative water content	Pandey and Pathak (2006)
Lolium perenne	Cr	Abnormalities in stomatal function and transpiration	Vernay et al. (2007)
Solanum lycopersicum	Pb	Limited tissue water content	Akinci et al. (2010)
Mesembryanthemum crystallinum	Cu and Zn	Decreased total water and relative water content	Kholodova et al. (2011)
Pluchea sagittalis	Pb	Decreased transpiration ratio	Rossato et al. (2012)
Ocimum basilicum	Cd	Reduced availability of water per unit area	Nazarian et al. (2016)
Zea mays	Cd and As	Reduced transpiration, stomatal conductance	Anjum et al. (2017)
Glycine max	As	Reduced root absorption and water content	Vezza et al. (2018)
Cinnamomum camphora	Zn and Cu	Reduced transpiration	Yang et al. (2018)
Citrus grandis	Cu	Reduced water uptake	Li et al. (2019)
Kandelia obovata	Zn, Cu, Pb	Transpiration and stomatal abnormalities	Shen et al. (2019)

types of mineral and HM. Nutrients with cationic potential will likely interact robustly with anionic HM. The newly formed complexes as HM and mineral nutrients interact could not lead to nutritional fulfillment of plants; hence nutritional deficiency occurs. Additional abnormalities in plants' functional activity are likely to happen when accumulation of HM in plants' tissues triggers homeostatic imbalance (Yadav 2010). Extreme heavy metal loads in plants often result in the production of reactive oxygen species and methylglyoxal which cause oxidative damage of lipids, proteins, and nucleic acids (Hossain et al. 2012). Roots and root hairs which

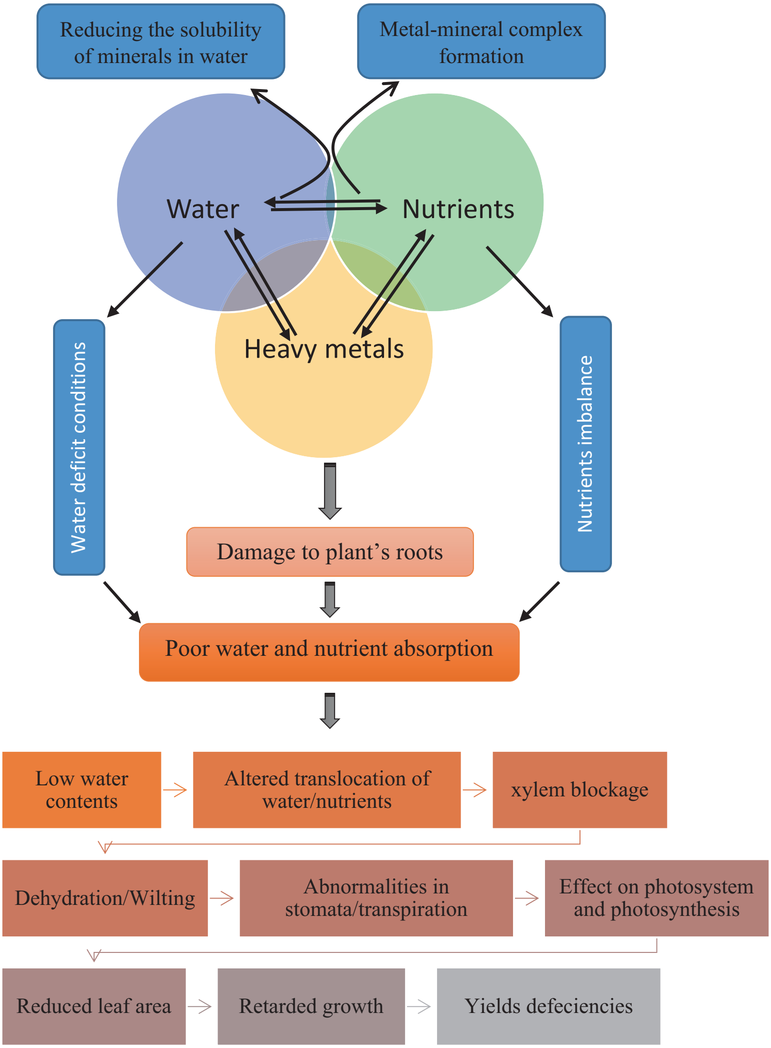

Fig. 1 A presentation of the heavy metal interactions with water and nutrient in soil and plants' tissues and their consequent effects on growth and physiology

are the primary contact points with soil substances upon exposures to HM are injured by the excessive amounts of HM or event by lower quantities of certain metals. Damage to cellular contents, leaves, and other organs becomes apparent in plants when they are encountered with HM (Singh et al. 2016). All these abnormalities lead to reduced growth of plants and production is limited substantially.

3 Photosynthesis Under Heavy Metal Stress

Photosynthesis is an important event in the life span of plants which contributes to food formation, storage, and growth increments. Several factors are linked with the initiation, proceeding, and inhibition of photosynthesis among which nutrients, water, cellular components, and availability of CO_2 are important determinants in the right direction of the process. Studies have shown that HM stress interacts in several ways with photosynthetic components (water, enzymes, CO_2, and nutrients). Rai et al. (2016) described that HM interacts with enzymes, chlorophyll biosynthesis, electron transport system, and enzymes involved in dark reaction. Najeeb et al. (2011) highlighted the structural abnormalities of chloroplast induced by Cd stress in *Juncus effuses*. In a study, reduced chlorophyll a and b and total chlorophylls were recorded in *Glycine max* when exposed to Ni stress (Sirhindi et al. 2015). Ali et al. (2015) documented a 27–45% decrease in chlorophyll contents and 84–71% increase in proline in *Linum usitatissimum* in response to elevated Cd and Cr concentrations. Mathur et al. (2016) observed reduced activity of electron transports and disturbance in photosystem II in wheat that were exposed to Cr stress. Ferreyroa et al. (2017) outlined that Pb-contaminated soil distorted chloroplast structure and chlorophyll pigments in *Brassica napus*. Recently, Lin and Jin (2018) reported that Cu contamination severely damaged photosynthetic pigments and gas exchange parameters in *Brassica* and *Chrysanthemum* species. Disturbance in photosystem II of wheat occurred at higher concentration of Zn and Cd in a hydroponic wheat culture (Paunov et al. 2018). These studies attributed damaged photosystems, pigments, and photosynthetic activities to the interactions of heavy metals with concerned enzymes and cellular components in green organs.

4 Effects of HM on Transpiration and Stomatal Activities

Transpiration in plants regulates nutrient and water uptake. The process is mainly controlled by proper functioning of stomata in leaves and other green organs. Adjustment of stomata in relation to the plants' conditions accordingly drives transpiration which stimulates physiological processes. HM occurrence in soil and their accumulation in roots, shoots, and leaves impart adverse effects on stomata and hence on transpiration. Reduced transpiration in plants caused by HM stress is linked with reduced stomatal density and aperture which in parts might be influenced by decreased hydraulic conductivity, leaf size, and reduction in intercellular spaces (Rucińska-Sobkowiak 2016). In barley, a reduced transpiration rate and stomatal conductance were observed when plantlets were exposed to elevated Cd and Cr concentrations (Ali et al. 2011). A mixture of HM, i.e., Cd, Cu, Cr, and Zn, resulted in altered pattern of transpiration in poplar (Chandra and Kang 2016). Stomata closure was recorded in *Salvinia minima* when challenged with excessive concentrations of Pb (Leal-Alvarado et al. 2016). In another study, Pb concentrations adversely affected net transpiration and photosynthesis in *Potentilla sericea* (Qi et al. 2018). The suboptimal activity of transpiration and other physiological parameters were recorded in Typha sp. in response to Ni stress (Akhtar et al. 2018).

5 Conclusion

Heavy metal occurrence in soils particularly in agricultural areas is a disturbing problem which has drastic effects on plants' physiology and growth. Human's activities and natural phenomena are the leading causes of HM pollution. Water and nutrient availability in soil and their absorption play key roles in major physiological events of plants. Heavy metal stress imparts negative effects on water and nutrient relation of plants. They cause water deficit condition and compete with nutrients for uptake. Absorption and accumulation of heavy metals result in physiological stress of plants and significantly lower their yield potentials. HM occurrence not only disturbs plants' water and mineral absorption but also drastically influences local population of beneficial microbes in soils which in one way or the other are associated with health benefits of plants. Their toxic effects on plants range from direct root injury to accumulation of heavy loads in living tissues which subvert basic processes necessary for growth. Blockage of xylem with HM results in wilting and translocation of food between sources and sinks. Other vital processes like opening and closing of stomata, transpiration, and photosynthesis are severely affected which reduces net photosynthate with subsequent impact on plant growth and yields.

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Heavy Metal-Induced Oxidative Stress and Related Cellular Process

Samir B. Eskander and Hosam M. Saleh

Contents

1 Heavy Metals

Toxic heavy metal designation is often applied to a group name for metals and semimetals (metalloids) that have been accompanied with contamination and massive toxicity or ecotoxicity (Duffus 2001). These include in addition to the transition

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metals some metalloids, lanthanides, and actinides (Singh et al. 2011). Their densities are relatively higher than water (Tchounwou et al. 2012). They are divided into essential metals of the kind as copper (Cu), cobalt (Co), iron (Fe), chromium (Cr), manganese (Mn), magnesium (Mg), selenium (Se), molybdenum (Mo), and zinc (Zn)). They are basic elements in the living systems, as most of them participate as cofactors for the enzymes involved in various biochemical as well as physiological functions required for cellular metabolism and growth (Tchounwou et al. 2012; Gutiérrez et al. 2015; Kim et al. 2015). The essential metals in nutrients participate, generally, in many essential vital biological processes such as nutrient absorption, gaseous exchange, respiration and redox reactions, CO_2 fixation, electron transfer, and structural functions in nucleic acid metabolism.

The others are nonessential metals, e.g., aluminum (Al), arsenic (Ar), nickel (Ni), mercury (Hg), platinum (Pt), lead (Pb), cadmium (Cd), titanium (Ti), and uranium (U), which imposed toxicity though at worthless concentrations (Tchounwou et al. 2012), Fig. 1. Heavy metals cause detrimental effects on all living organisms (Pudpong and Chantangsi 2015), leading to major ecological disturbances (Gutiérrez et al. 2015). Massive concentrations of the heavy metals are distinguished to be toxic referring to their non-biodegradability (Gutiérrez et al. 2015; Martín-González et al. 2006; Somasundaram et al. 2018).

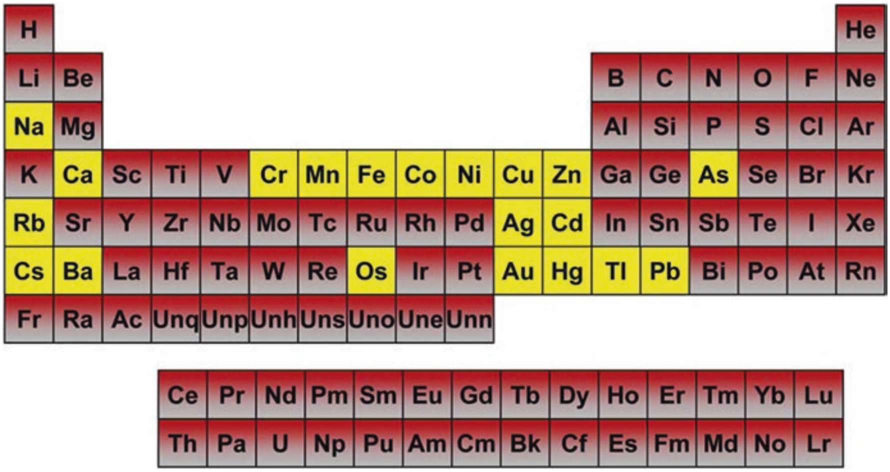

Fig. 1 The most studied metals inducing oxidative stress potential and their location in the periodic table. They are 20 alkalis, and transition and heavy metals assayed in cell lines, and are marked in yellow. Adapted according to Simmons et al. (2011)

For the most, phytotoxicity is a factor of bioavailability of those heavy metals in the ecosystem and their interactions with other metals, nutritional status, age, and mycorrhizal infection of the plant, as stated (Påhlsson 1989).

2 Sources of Heavy Metal

The massive pollution all over the world is referred to many reasons among them: Overpopulation, urbanization, affluence where the per capita consumption is very high and people discard many items regularly, technology i.e. the change in the culture of using things, and other causes (Chadar and Keerti 2017).

Heavy metal toxicity is expanding all over our world that adds more burdens and risks to man health and to his environment. Heavy metal pollution is a consequence of the release of the pollutants into the air, water, or soil as a result of man anthropogenic activities, either by accident or by undertaking. Heavy metal pollution can be defined like any other kind of pollution, the discharge of something unwanted into the environment. There are two widespread sources of heavy metal pollution, namely the natural and the anthropogenic sources.

2.1 Natural Sources

Small amounts of many toxic heavy metals from the earth's crust are contained in mineral springs, sand mounds, and volcanic eruptions due to soil erosion and natural weathering of the earth's crust.

2.2 Artificial Sources

The anthropogenic human activities add significant concentrations of heavy metals to the natural one. These activities have given rise to the spreading of many toxic pollutant metals from the earth's crust to various environmental compartments. Therefore, ecosystems are hazarded to heavy metals from various sources including mining and refining of ores, fertilizer and pesticide applications, battery manufacture, chemicals, disposal of solid wastes (including sewage sludge treatment), irrigation with wastewater, vehicular exhaust emissions, and most industrial activities (Teklić et al. 2008; Shahid et al. 2014) and to lesser extent from urban runoff, boating activities, and domestic garbage dumps (Pinto et al. 2003).

Wundram et al. (1996) notified that phytotoxicity constraining the photosynthesis in the green alga *Chlamydomonas* is due to the heavy metal pollutants percolating from salt mine wastes (Wundram et al. 1996).

The accumulation of toxic heavy metals in the ecosystems due to the human activities is, frequently, greater than the accumulation of those released from natural sources (Zalups and Ahmad 2003).

However, it is unobtainable to survive in an environment free of pollution with heavy metals. Yet, the main issue for mankind today, which seems to be a miracle, is creating pollution-free and clean ecosystems.

3 Factors Influencing the Heavy Metal Bio-uptake and Their Entry into Plant Cells

When a normal content of heavy metals is existing in soils, plants have the potential to avoid their hazard impacts (Van Ho et al. 2002). On the other side, tremendous amounts of heavy metals in the ecosystem cause harmful impacts on cellular and vital biological processes in plants (Ma 2005; Dimkpa et al. 2009).

Heavy metal bio-uptake by plant is not, always, in a linear relationship to its increasing concentrations. Many factors can influence the bioabsorption of metals by plants and those comprise the growing environment, such as temperature, soil pH, soil aeration, Eh condition (particularly of aquatic environment) and fertilization, competition between plant species, and type of plant, besides its size, root system, availability of the elements in the soil or foliar deposits, type of leaves, and soil moisture, in addition to the plant energy supply to the roots and leaves (Nagajyoti et al. 2010).

Fluctuations in soluble metal ion contents and their species in spiked soils significantly affect the metal bio-uptake by plants. The growth of either Indian mustard (*Brassica juncea*) or sunflower (*Helianthus annuus L.*), in long-term spiked soils, influenced pollutant bio-uptake by the two nominated plants. It was reported that the bio-uptake of the dissolved Cd and Zn decreased post-growing Indian mustard, and this was attributed to the elevation in the soil pH after the plant growth. Also, concentrations of soluble Cu and Pb diminished in acidic soils but increased in alkaline soils. This can be explained on the basis that the hyperaccumulator plants have been directed to either acidify rhizosphere environment in the spiked soils, therefore, increased the availability the dissolved concentrations of heavy metals or by elevating the soil pH after plant outgrowth. Increase in the soil pH and its dissolved organic carbon (DOC) content interacted in a competing way. In acidic soils the impact of pH was greater than that of DOC augmentations, leading to a total decrease in dissolved metal pollutant contents in these soils. In contrary, the increase in DOC content post-plant outgrowth increase dissolved metal concentrations in the alkaline soils. Chemical alterations in the rhizosphere, therefore, played an important role in controlling the speciation of available metal in soil solution (Kim et al. 2010).

Many postulated mechanisms have been put forward to describe the entry and the distribution of the toxic heavy metal ions, after their accumulation, inside the biological cells. Heavy metals cross the cell membranes based on their chemical and physical states besides their essentiality to the plant.

- Molecular mimicry can describe anakin mechanism, where the metals, in general, are contend for binding to multivalent ion carriers, e.g., Ca^{2+}.
- Post-binding to low-molecular-weight thiols, like cysteine, metal ions use amino acid transporters and cross the cell membrane through an active transport mechanism.
- Metals can, also, enter the cell by endocytosis mechanism when they are fastened to chelating proteins.
- Induction mechanism is based on the two specific polypeptides, namely metallothioneins (MTs) and phytochelatins (PCs). Both proteins are cysteine-rich polypeptides and have the capability to tie to metal pollutants through their sulfhydryl groups. It had been reported that metal binding by PC is mainly specific for cadmium (Cd), since the PC-deficient mutants are sensitive to Cd and not to other metals such as Cu, Hg, Zn, or Ni (Ha et al. 1999), while induction with MT involves transcriptional control mechanisms.
- Additional assumed mechanism is as follows: heavy metal pollutants can motivate membrane depolarization, followed by acidification of the cytoplasm (Cumming and Taylor 1990; Cardozo et al. 2002; Conner and Schmid 2003). The membrane harm is, foremost, the impact of high metal ion concentration which, usually, is terminated by disruption of cellular homeostasis, Fig. 2.

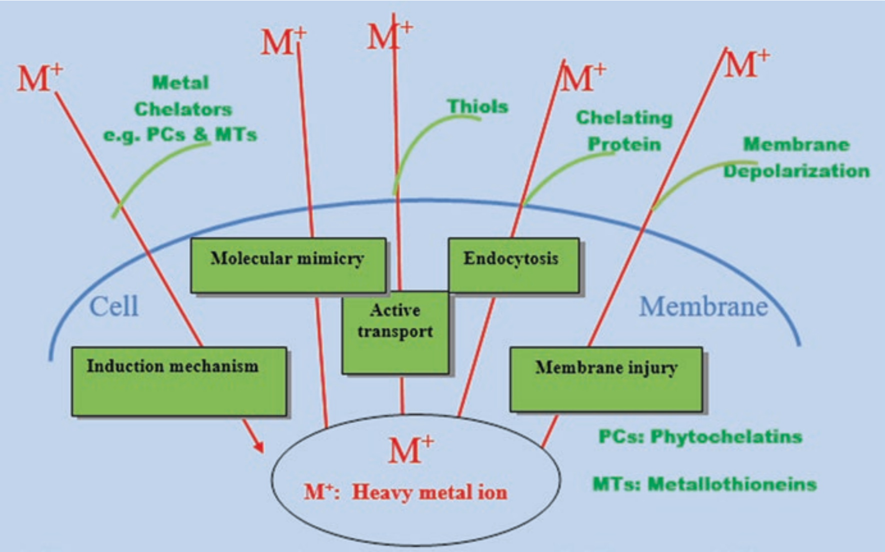

Fig. 2 Proposed mechanisms for entrance of heavy metal ion inwards the plant cell

4 Oxidative Stress

"A condition in which oxidation, due to reactive oxygen species exceeds the antioxidant tools in the living systems, past to a loss of the balance between them in favour of the former" can be defined as an oxidative stress. In a case when the active oxygen species, and consequently the free radicals, are generated intensively or at abnormal sites, the balance between formation and removal of ROS is disturbed, inducing a harmful oxidative stress. Hence, active oxygen species and free radicals can attack molecules in biological membranes and tissues. This led, mainly, to lipid peroxidation, oxidative DNA damage, protein and amino acid oxidation, physiologic alteration phenomena, and uncontrolled intracellular signal transduction.

However, oxidative stress is actually useful in some instances. ROS produced during normal cell metabolism has been shown to function as a key regulator in various physiological processes, such as seed germination, plant maturation, and senescence and stomatal closure (Yuan et al. 2013).

Reactive Oxygen Species (ROS) Heavy metals are toxic at tremendous concentration and their toxicity can be attributed to the production of reactive oxygen species (ROS) and, as a consequence, an unbalanced cellular redox status.

An oxygen molecule (O_2) undergoes four-electron reduction when it is metabolized in vivo. Due to this process, reactive oxygen metabolites are generated by the excitation of electrons secondary to addition of energy or interaction with transition elements. The reactive oxygen metabolites produced are more highly reactive than the original oxygen molecule and are called reactive oxygen species (ROS). Superoxide ($O_2^{\bullet-}$), hydroxyl ($HO^{\bullet}$), and singlet oxygen ($^1O_2$) as free radicals while hydrogen peroxide H_2O_2 as nonradical are the most familiar active oxygen species (Yoshikawa and Naito 2002).

The following is a very brief description of the formation and the role of the most active ROS (Sies 1985; Docampo 1995).

Superoxide Anion ($O_2^{\bullet-}$) This is one-electron reduction state of oxygen (O_2), generated in many autoxidation reactions as well as through the electron transport chain. It can release Fe^{2+} from iron-sulfur proteins and ferritin. It is a predecessor for metal-catalyzed $OH^{\bullet}$ and undergoes dismutation to H_2O_2 spontaneously or by enzymatic catalysis. The $O_2^{\bullet-}$ is a free radical that has a short biological life span due to its fast reduction to H_2O_2.

Hydrogen Peroxide (H_2O_2) It is a two-electron reduction state of O_2 and is formed by dismutation of superoxide anion or by direct reduction of oxygen. It is lipid soluble and therefore has the capability to diffuse across cell membranes. Hydrogen peroxide is a nonradical ROS with a long biological life span and greater steadiness relative to free radicals.

Hydroxyl Radical ($OH^{\bullet}$) It is a three-electron reduction state of oxygen, a product of Fenton reaction and decomposition of peroxynitrite. It is highly reactive of all ROS with half-life <1η second, and can attack most cellular components.

Singlet Oxygen ($^1O_2$) Metals without redox capacity such as cadmium, lead, zinc, and nickel can generate the singlet oxygen species. This ROS radical is able to produce superoxide ($O_2^{\bullet}$) (Fryzova et al. 2017). It is worth to state that carotenoid pigments can act as a chemical quencher for singlet oxygen free radical.

Reactive oxygen species (ROS), as stated, are spontaneous by-products of cellular oxidative metabolism and perform valuable tasks in cell signaling, modulation of cell survival, cell death, and inflammation-related factor generation. Biologically significant ROS elements, in addition to the most reactive presented ones, also include free radicals like peroxyl ($RO_2^{\bullet}$), carbonate ($CO_3^{\bullet -}$), carbon dioxide radical ($CO_2^{\bullet -}$), and alkoxyl ($RO^{\bullet}$), withal nonradicals, such as ozone (O_3), peroxynitrate (O_2NOO^-), hypobromous acid (HOBr), hypochlorous acid (HOCl), nitric oxide (NO), organic peroxides (ROOH), peroxynitrite ($ONOO^-$), peroxomonocarbonate ($HOOCO^-$), hypochlorite (OCl^-), and peroxynitrous acid (ONOOH), (Abdal Dayem et al. 2017).

5 Main Sources for Reactive Oxygen Species (ROS) Generation

Collectively, ROS are generated via both the extracellular and intracellular sources. The extracellular quarries of ROS are be the environmental pollutants, heavy metals at extremely high concentrations, radiation exposure, and microbial infection. On the other hand, intracellularly ROS can be originated from the mitochondria, cellular-metabolizing enzymes, endoplasmic reticulum (ER) stress, peroxisomes, microsomes, and nicotinamide adenine dinucleotide phosphate oxidase (NOX: NADPH) (Trachootham et al. 2009; Thannickal and Fanburg 2000).

Definitely, mitochondria considered the main intrinsic cascade for ROS generation via the mitochondrial electron transport pathway. Massive accumulation of calcium ions (Ca^{2+}) in the cytoplasm leads to stimulation of the mitochondrial electron transport link and, consequently, the ROS production. Small fraction of O_2 molecules is produced during the mitochondrial production of adenosine triphosphate (ATP) and water which represent the advance stages of ROS generation (Finkel 2012).

The superoxide anion ($O_2^{\bullet -}$) is the first ROS created by mitochondria, and stemmed from complex I (NADH ubiquinone oxidoreductase) and complex III (coenzyme Q, bc1 complex, and ubiquinone/cytochrome c reductase) activities in the mitochondrial matrix and intermembrane space, respectively (Dikalov 2011; Tahara et al. 2009).

In the intermembrane space, metals superoxide dismutase, of Mn, Zn, and Cu can catalyze the transformation of superoxide anions into H_2O_2 (the most stable ROS). Monoamine oxidase and α-ketoglutarate dehydrogenase are also powerful cascades for mitochondrial ROS production. NOX represents a non-mitochondrial source of ROS generation and takes a determined part in superoxide formation through oxygen reduction mediated by the electron donor NADPH. The ER is a cellular organelle that also performs an essential work in ROS production. The ER lumen represents a suitable oxidizing environment (with a high ratio of oxidized-to-reduced forms of GSH) for protein folding and formation of disulfide bonds. Additionally, there are various cellular enzymes, including cyclooxygenase, lipoxygenase, xanthine oxidoreductase, and nitric oxide (NO) synthase, implicated in the process of ROS generation. $ONOO^-$, which is considered a power oxidizing and nitrating agent, originates from interaction between NO and $O_2^{\bullet -}$.

Alongside, the extracellular source of ROS generation comprises ROS-inducing agents, e.g., radiation, and heavy metal pollutants. Most published works describe the role of iron in the Fenton reactions, which participates in the production of hydroxyl free radicals; iron ion (Fe^{2+}) is a key factor related to toxicity prompt by ROS generation (Abdal Dayem et al. 2017).

Copper (Cu) is an essential micronutrient, needed for most of the biological processes of life, as a cofactor for many enzymatic activities, charring some important physiological processes, e.g., respiration and photosynthesis. Cu acts specifically in phytosystem electron transfer components, leading to a massive production of ROS, and consequently the peroxidation chain reactions including the membrane lipids. Fe and Cu are both redox-active metals which directly induce ROS generation via Fenton and Haber-Weiss reactions (Schutzendubel and Polle 2002; Halliwell 2006).

According to Somasundaram et al. (2018) metals with redox activity like Cu generate ROS directly by autooxidation, as articulated, while metals without redox activity, like Cd, generate ROS indirectly by impairing the antioxidant defense mechanism (Somasundaram et al. 2018).

6 Production of Reactive Oxygen Species (ROS) Through Respiration and Photosynthesis

Some of ROS, e.g., superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), singlet oxygen (O_2 ($^1\Delta_g$), and hydroxyl radical (•OH), are found transiently in aerobic organisms. These species are normal by-products of oxidative metabolism and assert a constant threat to all aerobic organisms. It is worth to state that some of them can act as important signaling molecules that alter gene expression and modulate the activity of specific defense proteins. However, at high concentrations all ROS can be highly harmful to organisms. ROS can oxidize proteins, lipids, and nucleic acids, which results in changes in cell structure and mutagenesis (Pinto et al. 2003).

Also, according to Pinto et al. (2003), the accumulation of ROS establishes a particularly severe threat to photosynthetic livings, since the common biological source of O_2 is the single-electron reduction of molecular oxygen by electron transport pathways (Pinto et al. 2003). In fact, with reference to the intense electron flux in their microenvironment, which also contains elevated oxygen and massive metal ion concentrations, the mitochondria and chloroplasts of these photosynthetic, as cell compartments, are considerably susceptible to oxidative injury.

7 Phytotoxicity of Heavy Metals

The main symptoms and physiological changes of heavy metal toxicity on plants at cellular as well as molecular levels have been manifested mainly on disturbance in seed germination, general growth reduction or inhibition, detectable morphological changes, instability in water and ion homeostasis, effect on transpiration, enzyme activity diversion, free radical generation, and inhibition of photosynthesis.

An example for the phytotoxic impact for one of the heavy metals had been stated by Lin et al. (2007), where Cd can cause growth inhibition and even plant death for several plant species (Romero-Puertas et al. 2002; Wójcik et al. 2005). The plant growth inhibition induced by Cd was probably attributed to its role in reducing the plant photosynthetic rate, its detrimental impacts on chloroplast replication, and cell division (Baryla et al. 2001), and its effect on chloroplast structure (Barcelo et al. 1988), as well as its function in the water-splitting apparatus of photosystem II and photosynthetic electron transport (Mallick and Mohn 2003).

Liu et al. (2018) added an additional pattern for the impacts of copper as a phytotoxic heavy metal (Liu et al. 2018). Plants have the capability to bio-uptake and bioconcentrate Cu^{2+} from the spiked soil via their roots, which further makes the bioabsorbed ions translucent to the shoot parts of plants through the xylem tube. At the end, these ions are deposited and hold in the cell walls, vacuoles, and Golgi apparatuses through membrane transporter carriers' mechanism (Luo et al. 2016). For a large number of plants, elevated amounts of Cu^{2+} are toxic, which can lead to acute toxicity symptoms, like severe root devastation and plant growth retardation (Hall 2002). Alaoui-Sosseâ et al. (2014) and Atha et al. (2012) published that copper ion stress can modify the ion distribution of calcium, potassium, and magnesium in the cucumber root and leaves, and also constrain leaf expansion and photosynthesis process (Alaoui-Sossé et al. 2004; Atha et al. 2012). Massive cupric ion is also found to enhance lipid peroxidation and promote potassium ion efflux in Arabidopsis seedlings (Murphy et al. 1999). For most plants elevated concentrations of Cu^{2+} are phototoxic, and can exert the toxicity symptoms stated previously (Liu et al. 2018).

7.1 Cellular Phytotoxicity of Heavy Metals

The impacts of highly Ni-spiked soil on plants exhibited distinctive behaviors, e.g., reduced seed germination, outgrowth reduction, and diminished transpiration and photosynthesis (Sreekanth et al. 2013). According to Potters et al. (2007), plants under the stress of abiotic conditions suffered a number of stress-induced phenotypes of the same kind as retardation in primary root elongation, massive growth of lateral roots, and extensive number of root hairs (Potters et al. 2007).

Macroscopic observations revealed some chlorotic leaves in plants treated with 500 ppm Ni ions. In the same trend, plants subdued to increasing concentration of Cu (200, 500, 1000 ppm) presented massive cellular damage levels. Zinc contamination resulted in highest levels of phenotypic damage, confirmed through smaller leaf size compared with control samples following treatment with 720 ppm Zn, while higher Zn concentrations resulted in severe growth inhibition and chlorosis/necrosis. Germination rates were similarly affected negatively in Ni-treated seeds (Georgiadou et al. 2018).

According to Léon et al. (2005), excessive contents of nickel chloride ($NiCl_2$) resulted in the lowest germination rate compared with some other nickel salts, e.g., nickel acetate ($Ni(CH_3COO)_2$), or nickel sulfate ($NiSO_4$) (Léon et al. 2005). Variation in the germination rate impacts was attributed to some nickel salts having specific power-binding sites and properties specified through their action. Hence, $NiCl_2$ is potentially not as easily absorbed by plant leaves as it is by seeds, resulting in mild effects on the physiological parameters present and altering cell homeostasis.

According to Georgiadou et al. (2018), and with respect to photosynthesis-related parameters, pigment content reduced significantly along with elevating concentration of Ni, Cu, and Zn ions (Georgiadou et al. 2018). Similarly, plants subjected to high concentration of Ni exhibited a decrease in quantum yield of primary photochemistry and in chlorophyll contents (He et al. 2012). Comparably, 2000 ppm of Cu ions affects bamboo plants and asserts degradation of chlorophyll and consequentially the chlorophyll content is reduced considerably (Li et al. 2013). Also, extraordinary content of Cu ion can inhibit photosynthesis (Hou et al. 2007). These outcomes were corroborated by Georgiadou et al. (2018), where successive decrease in physiological processes was monitored through SPAD unit measurements in heavy metal-treated basil plants suggesting that heavy metals can disrupt photosynthetic parameters and photorespiration, changing the normal homeostasis of cells (Georgiadou et al. 2018).

It should be notified that clear indications of cellular damage were reported through significant increase in malondialdehyde (MDA) content in basal plants treated with 1000 ppm Cu ions, representing maximal cell membrane damage. In addition, the plant exhibited high H_2O_2 content in leaves at the same concentration of Cu ions (Georgiadou et al. 2018).

Elevated quantity of heavy metals (i.e., Cu or Fe) contributes to the production of HO^- from O_2^- through the Fenton reaction. Detected high level of MDA suggested that the metal ions augmented free radical accumulations (Choudhary et al.

2007). Related research proposed that massive content of Cu ions enhances free radicals, which in turn can cause nonenzymatic scission of cell-wall polysaccharides resulting in cell-wall loosening (Fry et al. 2002).

7.2 *Molecular Phytotoxicity of Heavy Metals*

Depending on the behavior of heavy metals in biological systems besides their chemical and physical properties, the metal phytotoxicity is attributed to either one of the following molecular mechanisms: (1) interference with functional sites in proteins and/or blocking of essential groups in biomolecules, e.g., suppressing enzymatic activities from their chelating to -SH groups on the enzyme; (2) disturbing enzymatic functions by displacement of essential elements from biomolecules; (3) stimulating the activity of NADPH oxidase; and (4) enhancement of ROS production directly through autooxidation by associating in biological redox reactions such as Haber Weiss and Fenton mechanisms (Sharma and Dietz 2009; Keunen et al. 2011; Schutzendubel and Polle 2002; Shahid et al. 2014).

Schutzendubel and Polle (2002) explained in some details the various molecular mechanisms for metal phytotoxicity that may be grouped as follows (Schutzendubel and Polle 2002).

(a) Generation of reactive oxygen species (ROS) through autoxidation and/or Fenton redox reactions: Under normal physiological conditions, heavy metals mostly exist as their positive cations. In addition, most of the heavy metals can be distinguished, biologically, to redox-active and redox-inactive groups. Therefore, metal cations which have lower redox reaction values cannot take part in biological redox reactions. According to this postulation, $O_2^{\bullet-}$ and subsequently H_2O_2 and OH• as ROS are generated from autoxidation of redox-reactive metal ions like Fe^{2+} or Cu^{+} through Fenton redox reaction.

(b) Blocking of essential functional group in biomolecules: This proposed mechanism for phytotoxicity is based on the capability of the heavy metals to bind to sulfur, oxygen, and/or nitrogen atoms in a biomolecule. This binding capacity attributed, mainly, to the free enthalpy and to the product development from heavy metal and ligand. According to this mechanism heavy metals can interfere specific enzyme function by binding to cysteine residues. Direct impact of cadmium on the sulfhydryl homeostasis of cells and suppuration of nominated enzymes had been reported (Chrestensen et al. 2000).

(c) Displacement of essential metal ions from biomolecules: Many heavy metals are basic part in lot of enzymes and are very essential for their activity. The displacement of a metal by its mimic can result in an inhibition of the enzyme function. For example, the divalent cations of Co^{2+} or Ni^{2+} or Zn^{2+} were reported to replace Mg^{2+} in ribulose-1,5-bisphosphate-carboxylase/oxygenase which led to loss of their activities (Van Assche and Clijsters 1986, 1990). Also, according to Rivetta et al. (1997); the displacement of Ca^{2+} by Cd^{2+} in protein calmodulin, which performs an important task in cellular signaling, resulted in constraining the enzyme activity in radish plants (Rivetta et al. 1997).

8 ROS and Oxidative Stress

Environmental stressors such as toxic heavy metals can create oxidizing imbalances in the cellular redox state resulting in a loss of reducing potential, a condition termed "oxidative stress" (Simmons et al. 2011).

ROS are produced during normal cell metabolism which act as key regulators for most of the physiological processes in plant as seed germination, plant maturation and senescence, and stomatal closure (Yuan et al. 2013). Besides their oxidative stress impacts, ROS can also act as a signal molecule for plants to sustain in harsh conditions such as elevated concentration of heavy metals. Cuypers et al. (2012) reported that mitogen-activated protein kinases (MAPKs) can perceive the changes of ROS content, owing to high accumulation of toxic metals in signal transmission for a range of stresses (Cuypers et al. 2012). The distinct MAPK pathways involved particularly in excess content of definite metal ions (Jonak et al. 2004; Liu et al. 2010; Arroyo-Serralta et al. 2005).

It is long established that heavy metals are powerful toxic agents that exert oxidative stress on biological systems through the provoking of reactive oxygen species (ROS), which overpower the cell's capacity to sustain its reduced status. The generated ROS can damage the cellular nucleic acids, proteins, and lipids, leading to the various cellular dysfunctions ending with cell death.

More generally, heavy metal toxicity is related, at least in part, to the oxidative stress induced in living systems. Heavy metals can promote oxidative damage both, directly, by increasing the cellular concentration of reactive oxygen species (ROS) and by reducing the cellular antioxidant capacity. On the contrary, the exposure to massive concentration of heavy metals can, also, stimulate detective response of the antioxidative tools. The direction of this feedback is a function, mainly, of the plant species, the pollutant inducing the phytotoxicity, mode of treatment, severity of the generated stress (Schutzendubel and Polle 2002), and surrounding ecosystem.

9 Plant Defense Mechanisms

Plants have multiple defense systems and complex regulatory mechanisms to overcome the hazard environmental conditions and avoid the excess metal-induced damages, such as regulating of metal ion uptake, chelation, perception transduction, and transmission of stress stimuli. Later publications have depicted that metal-deliberate stress has a dispose effect on massive generation of reactive oxygen species (ROS), amount of nitric oxide (NO), and hormone levels; consequently, the plants can rapidly acclimatize their outgrowth to the harsh conditions by balancing the cellular redox state and hormone signaling.

There are several strategies that plants can follow to survive with harsh tremendous concentrations of heavy metals in their spiked ecosystems. The first one is avoiding the bio-uptake of heavy metals into plant roots. Heavy metals can be leached out by plants by mycorrhizal association, metal sequestration, or complexation by exuding organic compounds from root system (Verbruggen et al. 2009; Antosiewicz et al. 2014). These mechanisms usually lead to immobilizing of the toxic heavy metals.

Heavy metals, at high concentration, are likely highly toxic to all living systems. Lot of research studies on the physiological responses to high concentration of heavy metal ions reported that plants have developed various mechanisms to counteract this environmental threat. Until now, however, the cellular mechanisms of heavy metal stress-induced signaling remained hard to describe and/or to understand. Essential and nonessential heavy metals have opposed physiochemical characters and roles in living systems. The former are vital micronutrients needed for plant growth and development. It is a cofactor for many physiological operations comprising photosynthesis, respiration, superoxide scavenging, ethylene sensing, and lignification. However, massive accumulations of these essential micronutrients are deleterious due to the generation of ROS by autooxidation reactions as previously stated. Nonessential heavy metals have no obvious biological function. It is highly reactive and inactivates various enzymatic pathways. Therefore, they are, generally, toxic for all living cells. Although they do not directly interfere with cellular redox reactions, it causes oxidative harm as a secondary effect. The displacement and thereby the release of redox-active metal ions from various biomolecules as well as the depletion of the antioxidant system by nonessential heavy metals disturb the redox balance of the cell.

Acclimatization responses of plants to heavy metal-spiked ecosystem are well-organized processes that comprise many physiological, molecular, genetic, and ecological pathways, which provide definite plant species the capability to survive or to hyperaccumulate the toxic metals (Sarma 2011).

As an alternative to keeping metals outside the cell, cells can also enhance the synthesis of protective proteins. Plants disclosed and elaborated defensive antioxidant tools to safeguard cells against pollutant stress. Enzymatic compartments of the antioxidant defense system comprise superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and ascorbate-glutathione pathway enzymes, which include ascorbate peroxidase (APX) and glutathione reductase (GR) (Fig. 3). Besides, there are a large number of nonenzymatic antioxidant compounds like ascorbate, glutathione, proline, carotenoids, and phenol compounds, which had been reported to be active in scavenging ROS (Mittler 2002; Aydoğan et al. 2017).

Mechanisms covering the heavy metal stress tolerance can be gathered into two groups: avoidance and tolerance strategies. The key ways, as stated, are based on retarding the bio-uptake of the metal pollutants, and consequently eliminating them from the tissue of the plant. This may take place through modification in pH of the

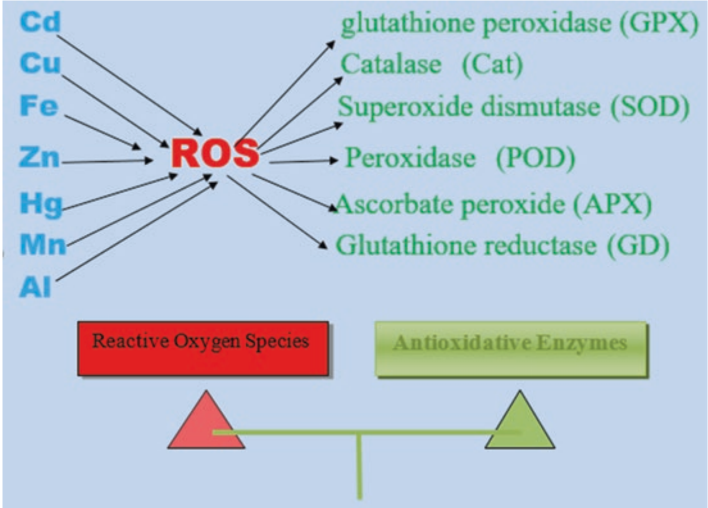

Fig. 3 Metals like Cu, Cd, Zn, Fe, Mn, Hg, and Al can prompt the reactive oxygen species (ROS) generation, and at the same time the ROS-scavenging systems, comprising superoxide dismutase (SOD), catalase (CAT), ascorbate peroxide (APX), peroxidase (POD), and glutathione reductase (GR), work to protect plant against the surplus ROS toxicity

surrounding rhizosphere, excretion of organic acids, and elaboration of mucous barrier on root tips or their stabilization in the cell walls (Hall 2002).

The second tolerance strategies can be explained on the capability of plants to accumulate, store, and stabilize heavy metals by tying them to chelator phytochelatins (PCs), proteins, peptides, or amino acids (Pál et al. 2006). It is postulated that the PCs chelate metal pollutants in the cytosol, and the formed PC metal complex is cleared in the vacuole.

Syntheses of metal-binding ligands, hormones, and/or growth regulators are among the numerous defense mechanisms against the toxic metal stress in plants. The metal chelator phytochelatins are the prime fields for heavy metal defense mechanism as published. Phytochelatins are synthesized, mainly, from γ-glutathione (γ-Glu), cysteine (Cys), and glycine (Gly), with a structure of $[(\gamma\text{-Glu-Cys})_n]$-Gly, where n is the number of repetition of the (γ-Glu-Cys) units. They are composed by the polymerization of glutathione units catalyzed in the presence of transpeptidase phytochelatin synthase. Glutathione (GSH), γ-glutamylcysteinyl glycine tripeptide, is the major cascade of nonprotein thiols in almost all plant cells (Bergmann and Rennenberg 1993). GSH participates in a crucial task in protecting plants from environmental stresses, including oxidative stress as a result of the generation of active oxygen species, xenobiotics, and some heavy metals. When GSH is oxidized as part of its antioxidant activity, it forms glutathione disulfide (GSSG). The glutathione reductases reduce GSSG back to GSH by utilizing reducing equivalents from NADPH. It is noted that, for example, two genes encoding GSH reductase have been identified in Arabidopsis plants; one, *gr2*, encodes a plastidic isoform (Kubo et al. 1993), and the other, *gr1*, encodes a cytosolic enzyme (Xiang and Oliver 1998).

As the disturbance of the homeostasis, the GSH was rapidly devastated in contesting the imposed stress. In response, plant cells have to restore the GSH balance by producing more GSH to sustain a high GSH/GSSG ratio. This is approved at various levels. First, the feedback retardation of γ-glutamylcysteine (γ-GC) peptide synthetase, immediately, by the decrease in GSH level, then, is restoring GSH synthesis by the preexisting enzymes. However, the GSH-synthesizing capability of preexisting enzymes can never significantly elevate without de novo enzyme synthesis. To meet the ever-increasing demand for GSH as imposed stress becomes harder, plant cells respond with high-steady-state mRNA accumulation, which is controlled by transcriptional activation and possibly by enhanced translation of preexisting mRNA. Consequently, enzymes are more generated through de novo protein synthesis, leading to an enhanced GSH-synthesizing capacity, thereby recovering GSH levels. There must be controls to switch these mechanisms on and off accordingly with the changing status of the stress. The identity of the signal molecule(s) and the nature of signal transduction are mostly unknown, although results by consuming that H_2O_2, the GSH, GSSG concentrations, and the GSH/GSSG ratio are not in the signal transduction pathway (Xiang and Oliver 1998). Therefore, accessibility of glutathione is very important for PC production in plants, at least during their exposure to tremendous content of the polluted metals (Yadav 2010).

Plant hormones and growth regulators both can activate the heavy metal-responsive genes and this can explain the postulated relationships between the growth regulator compounds and the synthesis of the phytochelatin at molecular or at gene expression level. Therefore, that detected link between hormonal pathways and metal-binding ligands, as one of the plant defense systems, may be illustrated on the basis of specific signaling pathway or common synthesis pathway (Pál et al. 2018).

The cellular mechanisms for exudation of chelating compounds and active efflux of metal ions, conventionally, take place by primary ATPase pumps (Rosen 1996).

In addition to the antioxidant nature of high protein molecule origin, which is outlined above, there are, also, a number of metal chelators like metallothioneins (MTs) and (PCs) (Cobbett and Goldsbrough 2002). Both chelators are based on cysteine-rich polypeptides and their binding activities are attributed to their capacity to coordinate the metals using the sulfhydryl groups in the polypeptide. However, PCs are short, from 5 to 11 amino acids long, and are formed by condensation of glutamate and cysteine via a pathway involving GSH. Contradictorily, MTs are synthesized by translation of mRNA and can be up to several hundred amino acids long. The promotion mechanisms also differ for the two classes of chelators (Steffens 1990). Excessive rate of PC generation needs the formation of a complex between GSH and a heavy metal, especially cadmium (II) ions. Therefore, newly formed PCs appear not to require new production of GSH-metabolizing enzymes. In contrast to the PCs, induction of MT involves transcriptional control mechanisms. For example, Cu has been observed to promote MT gene expression in the

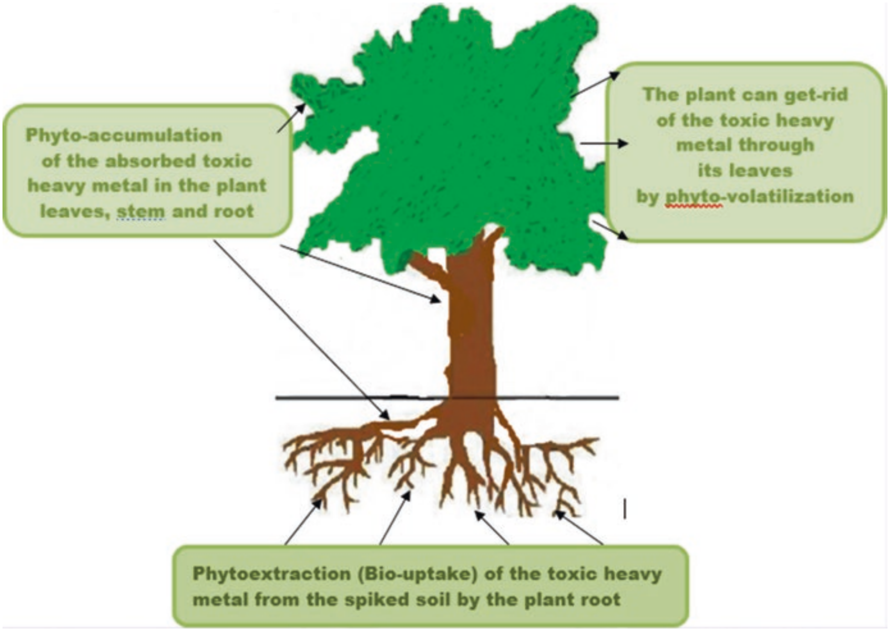

Fig. 4 Various processes involved in phytoremediation of heavy metals. Phytoextraction, phytoaccumulation, and phytovolatilization are the main mechanisms postulated to be proceeded in the accumulation process of a toxic heavy metal by plants (Yang et al. 2002)

seagrass *Posidonia oceanica* (Giordani et al. 2000) and the brown alga *Fucus vesiculosus* (Morris et al. 1999). It is also possible that heat-shock proteins may have a task in the cellular defense, although this cannot be referred to a metal-chelating activity (Vierling 1990).

Mechanisms to remove the highly accumulated reactive active oxygen species in the living cell are elementary to sustain its life. Therefore, numerous antioxidant defense performances are acquired during the progress of evolution. In case, the ROS or free radicals are formed massively or at abnormal sites; the balance between their generation and their scavenging is disrupted, leading to oxidative stress (Figs. 3 and 4).

Plant cells have extended defense systems to oppose the impacts of oxidative stress by scavenging free radicals, which are induced by the oxidizing agents, and repairing the resulting damages to biomolecules. The activation of this antioxidant stress-response state is a dependable indicator of oxidative upset.

Hg has negative effect on alga growth and stimulates ROS production. To avoid the Hg-induced oxidative stress, antioxidative enzymes such as SOD, CAT, and APX are released to regulate the redox balance of the cell by diminishing the excess ROS.

A comparable impact for Cd heavy metal stress was also reported, where the enzyme activities of CAT, APX, and GR are elevated in both plant roots and leaves as a result of excessive Cd exposure. Also, APX activity was declined in the roots of the plants under Cu stress. In addition, the antioxidative enzyme activities are differentially balanced in roots and leaves after excess Cu exposure; for example CAT activity is decreased in roots, but increased in leaves. Besides the impact of oxidative stress, ROS can act as a signal molecule for plants to survive in harsh heavily polluted conditions such as metal stress. It has been documented that mitogen-activated protein kinases (MAPKs) can perceive the alterations in ROS content in signal transmission for a range of stresses and the distinct MAPK pathways involve specifically excess particular metal ions. ROS induced by Cu triggers SIMK and SAMK activation via SIMKK, whereas Cd activates MMK2 and MMK3 besides SIMK and SAMK. Recently, MPK3 and MPK6 are also evidenced to be activated by Cd-induced ROS accumulation in *Arabidopsis* plants. A similar effect is also shown in plant response to Al stress, in which a MAPK-like protein is modulated by toxic concentrations of aluminum (Liu et al. 2010; Arroyo-Serralta et al. 2005).

10 Plant Growth Regulators

Multiples of plant hormones and plant growth regulators are found to be associated with heavy metal stress responses. The detected link between hormonal pathways and metal-binding ligands, as one of the plant defense systems, can be explained on the basis of specific signaling pathway or common synthesis pathway (Pál et al. 2018).

Reactive oxygen species stress, due to high concentration of heavy metal, induces disruption in the metabolism of plant hormones or plant growth regulators. These alterations participate in the perception and responses to ROS stress. Many studies on the relationship between plant growth regulators and thiol-related peptide synthesis had been reported and some examples are described hereafter.

10.1 Abscisic Acid (ABA)

ABA is a characteristic stress hormone, controlling many essential processes in plants (Vishwakarma et al. 2017). Many toxic metals can induce ABA synthesis. High concentration of arsenic compounds leads to increase in ABA biosynthesis genes as well as upregulation of ABA signaling genes in rice (Huang et al. 2012). Exogenous abscisic acid reduced Cd content and consequently enhanced Cd tolerance in rice (Hsu and Kao 2003). ABA affected the GSH content, GSH:GSSG ratio, and γ-glutamylcysteine synthase (γ-ECS) transcript level in two maize genotypes differentiating their stress tolerance (Kellős et al. 2008), and vice versa GSH treatment also enhanced ABA synthesis and accumulation (Cheng et al. 2015) suggesting a relationship between GSH and ABA.

10.2 Auxin

Auxin is a growth hormone, which stimulates plant growth and enhances the response to gravity or light stimuli (Zhao 2010).

Its synthesis, dispersion, metabolism, and circulation in the plant are controlled by various environmental factors and can be disrupted by elevated content of metal pollutants (Hu et al. 2013). Farooq et al. (2015) reported that addition of tryptophan, a precursor of auxin, to the roots of rice seedlings enhanced plant growth and yield under cadmium stress. Eckardt (2010) published that the level of endogenous GSH affected the expression of auxin transport gene. Also, increased hormone level was accompanied with an increment in the glutathione-*S*-transferase activity in the roots.

Therefore, auxin hormone participates in growth regulation as well as heavy metal resistance, besides its crucial function in signaling (Bouzroud et al. 2018). It is worth to denote that when the *Arabidopsis* seedlings were exposed to oxidative stress-inducing agents, changes in auxin homeostasis with physiological responses were monitored, assuming a possible interaction between ROS and auxin signaling. This observation is further confirmed based on experimental data where the increased ROS can systemize auxin distribution via altering the expression of *PIN* genes, reposition of auxin exporters (Grunewald and Friml 2010), and auxin conjugation (Tognetti et al. 2010). In addition, H_2O_2 can activate a specific *Arabidopsis* MAPKKK, ANP1, which can suppress auxin signaling (Kovtun et al. 2000). Tsukagoshi et al. (2010) reported that ROS controls the transition from cell proliferation to differentiation in roots through a separate pathway different from auxin signaling (Tsukagoshi et al. 2010). Also, it is dedicated that increased H_2O_2 in Cu-treated seedlings does not relate to Cu-regulated auxin redistribution, and consequently the retardation of primary root elongation (Yuan et al. 2013).

10.3 Cytokinins (CKs)

Cytokinins were described to be incorporated in, nearly, all aspects of plant growth, and also CK-dependent modulation in stress responses (Raines et al. 2016). A decrease in CK content in wheat plants was reported post-Cd treatment, which was accompanied with elevation in CK oxidase (Veselov et al. 2003).

According to Al-Hakimi (2007) and Piotrowska-Niczyporuk et al. (2012), it has been noted that exogenous CKs were able to counteract the induced toxicity of heavy metal in various plant species as a result of enhancement in antioxidant capacity (Singh and Prasad 2014). Furthermore, plants' overexpression of cytokinin oxidase/dehydrogenase 1 showed increased expression of genes related to PC biosynthesis (Mohan et al. 2016). Recently it has, also, been stated by Bruno et al. (2017) that Cd-induced inhibition of root growth may be related to an altered homeostasis of auxin/CK signaling.

10.4 Ethylene

It is a volatile compound that mediates many processes of plant growth and adaptive responses to a various stress (Chang 2016), including heavy metal stress. Keunen et al. (2016) reported that many pollutants induced ethylene synthesis and signaling in different plant species (Keunen et al. 2016). Generally, induction of ethylene by metals may cause unidentified symptoms in plants but has a role in Cd-induced cell death. However, a certain accumulation of ethylene could lead to an increase in sulfur metabolism and GSH synthesis (Masood et al. 2012; Thao et al. 2015).

10.5 Melatonin

Melatonin is rather a plant growth regulator (Arnao and Hernández-Ruiz 2007; Li et al. 2017). Cd stress has been found to increase melatonin content in rice (Byeon et al. 2015); in addition, melatonin treatment mitigates Cd stress through the induction of PC synthesis in tomato (Hasan et al. 2015).

It had been published that melanin has the capacity to change the biochemical pathways in fungal cells of *Aspergillus niger* when exposed to radiation stress due to Cs-137 and Co-60 radioisotopes (El-Sayyad et al. 2018).

In addition to the stated examples, there are many other plant hormones or growth regulators such as salicylic acid (SA), gibberellins (GAs), brassinosteroids (BRs), jasmonic acid (JA), and methyl jasmonates (MeJA) which are assumed to be engaged in plant growth and in response to heavy metal stress (Pál et al. 2018).

In general, plants are able to protect themselves from toxic metals. This derived from their capability to extract, accumulate, distribute, and immobilize those metals into their body for a long time (Fig. 3). Therefore, phytoremediation approach for remediating high concentration of hazard metals based on the aid of plants to reclaim spiked soils and water resource is a preferable new technology and very-low-cost choice, in order to have a safe, tide, and sustainable environment (Saleh et al. 2017, 2019; Saleh 2012; Eskander et al. 2011a, b).

11 Conclusion

Tremendous concentrations of toxic heavy metals actually have threatened impacts upon various physiological and biochemical processes in living organisms, putting markedly human health and his/her surrounding environment in major risks. Numerous research-based studies have established the danger of elevated toxic metal exposure for human health. However, it is worth to mention that the accumulation of those toxic metals and their ions into crop plants, and then the uptake of those hazardous elements via the food chain, puts forward man and animals on alarming risk.

Numerous biotechnological efforts are performed to improve tolerance and capability of plant in defiance of metal toxicity as well to apply it to bioremediate toxic materials including radioactive ions and heavy metal in spiked soil and water streams. This new, environment-affable, achievement is known as phytoremediation and based on the plant efficiency for detoxification and homeostasis of harm ingredients in its ecosystem.

For more progress in this approach, it is noteworthy to understand cellular signaling induced by the pollutant ion stress.

Again, supposing it is unobtainable to survive in an environment without heavy metal pollutants, yet, the main issue for mankind today, which seems to be a miracle, is creating pollution-free and clean ecosystems. The phytoremediation approach for remediating heavy metals based on the aid of plants to reclaim spiked soils and water resource is a preferable and very-low-cost choice, in order to have a safe and sustainable environment.

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Molecular Mechanisms of Heavy Metal Tolerance in Plants

Nasser Delangiz, Bahman Khoshru, Behnam Asgari Lajayer, Mansour Ghorbanpour, and Solmaz Kazemalilou

Contents

1 Introduction

The physiology and molecular response of plants are interesting as plants as sessile creatures are exposed to constant environmental harsh conditions which can affect their growth and productivity negatively. Among adverse effects heavy metals' (HMs) impact is a crucial one which can affect plant physiology and metabolic pathways (Asgari Lajayer et al. 2019a). Heavy metal term is used for metals with a determined specific gravity (>5); however biologists use this term for various metal(loids) that are toxic for organisms. Arsenic (As), cadmium (Cd), calcium (Ca), cobalt (Co), iron (Fe), manganese (Mg), nickel (Ni), and zinc (Zn) are some

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of the important HMs (Asgari Lajayer et al. 2017a). Some of metals such as Ca, K, Na, Mg, Fe, and Zn are known as essential elements as they are involved in the proper function of different proteins in organisms. So they are vital for growth, development, and healthy life of all organisms on the planet. However, their concentration is vital and above a normal range they can be toxic (Asgari Lajayer et al. 2017b). HMs naturally exist in soil and water; however their contamination becomes one of environment experts' concerns recently. The challenge of HMs becomes worse and worse because of human's destructive industries (Asgari Lajayer et al. 2018). This leads experts to be concerned of the potential of various organisms, such as plants, to ameliorate HMs' negative effects on the environment. The entry of HMs into the human food chain is so dangerous which can show its detrimental effects in the future (Asgari Lajayer et al. 2019b). So, unraveling the complexity of HM phytoremediation can help experts to develop some efficient eco-friendly agents to decrease the negative impacts of HMs on the environment (Asgari Lajayer et al. 2017a, 2019a).

HMs have various range of negative effects (from weak to moderate to strong) on plants based on their oxidation states. The molecular and cellular levels of HM toxicity include protein and enzyme denaturation, functional group blocking, essential metal substitution, and membrane disruption (Rascio and Navari-Izzo 2011). All these modifications can result in photosynthesis inhibition and respiration (Fig. 1) (Ali et al. 2013). Methylglyoxal (MG) is the other toxic compound which has been identified recently to increase under various stresses (Ali et al. 2013; Sytar et al.

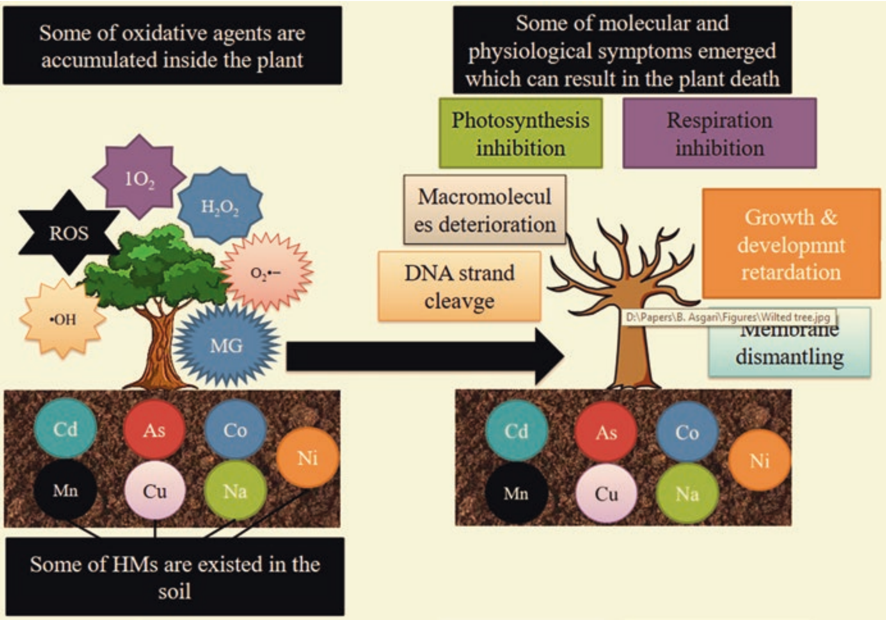

Fig. 1 The negative effects of heavy metals on plants

2013). The production of MG can interfere with plants' different mechanisms such as antioxidant defense system and photosynthesis that may eventually result in intensifying the generation of ROS (Dalvi and Bhalerao 2013; Viehweger 2014). Oxidative stress can deteriorate macromolecules (such as proteins), dismantle the membranes, cleave DNA strand, and finally demise the plant (Fig. 1) (Flora et al. 2008; John et al. 2012).

Changing physiological and biochemical processes, such as global gene expression, plants can develop their tolerance to stresses (Manara 2012). Plants use different strategies—avoidance and tolerance—to deal with the metal(loid)s. When the plant is able to restrict the absorption of metal(loid)s, it uses avoidance strategy. The plant does this by biosorption to cell walls, extracellular precipitation, reduced uptake, and increased efflux. However, when the plant is able to survive under high concentration of metal(loid)s, it uses tolerance strategy. The plant does this by chelating through amino acids, GSH, metallothioneins (MTs), and organic acids, or through compartmenting with vacuoles, and antioxidative defense mechanism upregulation (Hatata and Abdel-Aal 2008; Ali et al. 2013; Bielen et al. 2013). In this review chapter we prepare a comprehensive up-to-date knowledge about the way of plants' response to HM stresses. However, our major focus is on the MG and ROS metabolism and their relation to GSH. We also inspect the gene function and its relation to the MG and ROS detoxification mechanisms which are induced under HM stress.

2 Toxic HMs and Their Action in Plants

The manifestations of HM accumulation in plant cells are deferent. Redox active—directly involved in redox reactions—and redox inactive—not involved in redox reactions directly—are two groups of HMs. The exposure of plant to the redox-active HMs (Co, Cr, Cu, Fe) directly results in the generation of $O_2^{\bullet-}$, $^{\bullet}OH$, and H_2O_2 through Haber-Weiss and Fenton reactions (Asgari Lajayer et al. 2017a). However if plants are exposed to the redox-inactive HMs some indirect mechanisms, such as electron transport chain disruption, lipid peroxidation induction, and antioxidant defense mechanism malfunction, cause oxidative stress. HMs can strongly bind to O, N, and S atoms and play their destructive roles (Sharma and Dietz 2009). Due to this strong affinity of HMs they can easily inactivate enzymes. For instance, Cd binds sulfhydryl groups and inhibits the activity of enzymes and function of structural proteins (Nagajyoti et al. 2010). Enzymes often need HM ions to do their duties in bioprocesses. They are known as cofactors. Cu^{2+}, Mg^{2+}, Fe^{2+}, and Ca^{2+} are some of the important cofactors in the cell. These cofactors have precise action in their related enzymes and their displacement leads to loss or inhibition of enzyme activity. For instance, substitution of Mg^{2+} in RuBisCO with each of Zn^{2+}, Ni^{2+}, and Co^{2+} leads to inactivity of the enzyme (Kumar and Maiti 2013). Calmodulin-dependent phosphodiesterase activity is inhibited through displacement of Ca^{2+} by Cd^{2+} in calmodulin in radish (Nematshahi et al. 2012). HMs also damage the membrane. They cause protein thiol oxidation or cross-linking in membrane, inhibit the function of H^+-ATPase, and alter the fluidity of the membrane (Asgari Lajayer et al. 2019a). Plants accumulate MG in response to HM stress which eventually results in oxidative stress due to GSH content reduction (Fozia et al. 2008).

The toxicity of HM is contributed to three major reasons: (a) The production of MG and ROS is stimulated by alteration in the defense mechanism against antioxidant agents. They can also be produced through auto-oxidation or Fenton reaction. (b) Essential metal ion substitution with other (heavy) metals. (c) Binding of HMs to functional groups (such as carboxyl, histidyl, and thioyl) because of their high affinity to these groups (Tangahu et al. 2011).

3 Plant Responses to HM Stress

Plants trigger various metabolic and physiological modifications under HM exposure (Ghorbanpour et al. 2016). However, the responses are different between various HMs, because different HMs have different places to act in plants. The reduction in plant growth is the most prominent visual symptom (Nath et al. 2008; Nagajyoti et al. 2010). Chlorosis and necrosis, decreased seed germination rate, and decrease in the photosynthesis efficiency are related to molecular and biochemical alterations which are brought about by HM stress (Živković et al. 2012). Also, water uptake, evapotranspiration, and nutrient metabolism may be influenced under HM stress (Vezza et al. 2018). HMs can interfere with the uptake of elements, such as Ca, K, P, and Mg (Asgari Lajayer et al. 2017b). Plants' exposure to HMs usually affects the structure and function of thylakoids, so it can affect the photosynthesis complex directly. HMs cause the damage of thylakoid membrane through the release of some components from it (Muszyńska et al. 2018). Moreover, Mg can be replaced by some HMs. Chlorophyll synthesis may be reduced due to the destructive effect of HMs on the enzymes involved in the synthetic pathways of it (Asgari Lajayer et al. 2019c). HMs may also inhibit the function of enzymes related to CO_2 fixation. Therefore, HMs may interfere with both photosynthesis and carbon assimilation processes and eventually cause plant death (Ghorbanpour et al. 2016). For instance, photosynthesis rate of *Zea mays* L. is decreased under Pb stress (Ahmad et al. 2011). Also, the respiration inhibition of *Oryza sativa* L. was reported under the exposure of cadmium (Llamas et al. 2000).

HM toxicity causes the accumulation of extra ROS in plant cell. Some HM metals can directly generate ROS, such as Cu. However, others do this indirectly. For instance, Cd inactivates enzymes through LOX expression induction which eventually results in fatty acid oxidation. This triggers the production of ROS inside plant cells (Skórzyńska-Polit et al. 2006). Although ROS causes cell disturbances, plants' antioxidative defense mechanism helps them to overcome stresses such as the ones from HMs (Asgari Lajayer et al. 2017a).

4 The Molecular Basis of HM Tolerance

The response of plant to various stresses, such as HM stress, is the result of physiological and molecular interrelationship network. Therefore, study of this network, each mechanism individually and their genetic basis, is important to introduce some new tolerant species (DalCorso et al. 2010). Different plants use different mechanisms to cope with HM stress. Adaptive and constitutive mechanisms are two main mechanisms to cope with excess amount of HMs in the environment (Meharg 1994). Experts use all physiological, biochemical, and molecular approaches to unravel the mechanism of accumulation, adaption, and tolerance of HMs in plants. Synthesis of various amino acids, signaling molecules, and proteins occurs in the molecular level in plants under stressful conditions. For instance, recent studies proved the accumulation of heat-shock proteins (HSPs) under HM exposure (Zhao et al. 2011). The higher proline accumulation was reported in *Solanum nigrum* L. (a Cd hyperaccumulator) compared with *Solanum melongena* L. (a nonaccumulator) (Sun et al. 2007). This can prove the role of proline in detoxification processes of HMs. Some chelating compounds such as nicotianamine, phytosiderophores, and organic acids are released by plant roots and can influence the uptake of HMs (Dalcorso et al. 2010). Mitogen-activated protein kinases (MAPK) are triggered under HM stress. They have various forms with different kinetics which are induced under different stress pressures. For instance, *Medicago sativa* L. showed the activation of four different MAPKs (MMK2, MMK3, SAMK, and SIMK) under Cu and Cd stress. Cu stress induced the production of MAPKs more rapidly than the Cd stress (Jonak et al. 2004). Plasma membrane exclusion, immobilization, HM uptake and transport restriction, HM chelation, stress protein upregulation, and production of polyamines and signaling molecules such as nitric oxide and salicylic acid have also been reported (Zhu et al. 2011).

4.1 HM Uptake and Transport Restriction

To take up HM ions from the soil plants intercept HMs by their roots, take them up by their roots, and translocate them to the shoots. The uptake of the HMs through apoplast or symplast depends on the type of HMs. Most of the HMs are taken up to the plant through energy-consuming processes (Shahid et al. 2017). Avoidance strategy is a method to cope with HMs employed by plants. Plants avoid to take up excess amount of HMs based on this strategy. Complexing and precipitation are two avoiding strategies. Precipitation occurs through increasing the pH of root environment or excreting some anions (such as phosphate). For instance, the excretion of phosphate was shown in the aluminum-tolerant maize cultivars. The tolerant cultivars (South American 3) did not show toxicity symptoms; however, sensitive cultivars (South American 5 and Tuxpeño) showed (Pellet et al. 1995). It was also shown that oxalate secretion from the root apex of tomato helps the plant to exclude Cd from the root environment (Zhu et al. 2011). These findings suggest that HM-binding agent's excretion into the root environment plays an important role in HM-avoidance mechanism of plants.

4.2 The Hyperaccumulation Mechanisms of HMs

Plants which tolerate HMs often use three strategies to cope with them. These plants may restrict the translocation of HMs from root to shoot, or retain the root cell uptake, or chelate them and eventually store them into the vacuoles. There are rare plant species which are able to transport HMs from the root to the shoot efficiently. This translocation occurs through xylems which is driven by transpiration force probably (Salt et al. 1995). These plants are able to accumulate HMs even in low concentration from the environment. The concentration of HMs in the cell membrane of hyperaccumulators is unusually high. This can occur because of the high expression of HM transporters in the cell membrane. These transporters were identified in *Thlaspi caerulescens*, a Cd and Zn hyperaccumulator (Lombi et al. 2001). *T. caerulescens* has highly efficient chelating and intracellular compartmentalization mechanisms (Pilon-Smits and Pilon 2002). It has low concentration of HMs in root vacuoles, high translocation rate of HMs from the roots to the shoots, and high uptake of HMs to the leaves.

4.3 Chemical Modification

HMs can be assimilated into the organic molecules through metal-modifying enzymes. Changing the oxidation state of HMs is the other detoxifying mechanism that occurs through metal-modifying enzymes. Dimethyl selenide is the organic and less toxic form of selenite. This modification occurs through methyltransferase. Cr (VI), the toxic form of Cr, may be modified by chromate reductase and Cr (III), the nontoxic form of Cr, can be produced. For instance, water hyacinth (*Eichhornia crassipes*) is capable of detoxifying Cr (IV) through root uptake. Indeed this plant can accumulate the nontoxic form of Cr in its root and transport some part of this Cr to leaf tissues. Dicots can reduce Fe through reductase enzymes in their root cell membrane before uptake (Pilon-Smits and Pilon 2002). Totally, HM reduction is a useful phytoremediation mechanism to detoxify HMs.

4.4 Transcription Factor (TF) Modulation

Metal response-binding transcription factor 1 has a major role in response to and tolerance of HMs, as it triggers the activation of genes responsible for the detoxification, transport, and uptake of HMs in plants. Various TFs involved in HM stress response are identified in different plants. For instance, basic leucine zipper (bZIP), myeloblastosis protein (MYB), WRKY, and ethylene-responsive factor (ERF), from different TF families, control the expression of some genes responsible for the Cd stress tolerance (Bielen et al. 2013).

4.5 Proline Synthesis Under HM Stress

The proline accumulation under HM stress has been reported frequently (Tangahu et al. 2011; Guo et al. 2012). The increased level of proline in plant can produce an enhanced protection against Cd (Islam et al. 2009). HM-tolerant plants have elevated proline content in comparison with the nontolerant plants in the absence of HMs (Tangahu et al. 2011). Proline does not have a role in sequestering HMs. But its role is in the reducing of HM-induced damaging of free radicals. It was shown that the HM tolerance of the *Vigna radiata* L. increased by applying exogenous proline, because it enhances the amount of GSH and GSH-metabolizing enzymes (Flora et al. 2008). However, further research towards integration of the growth-inhibiting and -protecting properties of Pro is needed. Huang et al. (2010) studied the physiological and biochemical responses in leaves of two mangrove plant seedlings (*Kandelia candel* and *Bruguiera gymnorrhiza*) exposed to multiple HMs (Cd^{2+}, Pb^{2+}, and Hg^{2+}) and concluded that Pro, GSH, and PCs-SH in *K. candel* may play a more important role in ameliorating the effect of HM toxicity than in *B. gymnorrhiza*.

5 Transgenic Plants and HM Tolerance

Molecular biology is a strong field to study and improve the mechanism of HM tolerance in plants, as it can reveal key steps in this mechanism through molecular approaches such as genetic engineering (Pilon-Smits and Pilon 2002). Many genes are identified through classic genetics involved in uptake, sequestration, tolerance, chemical modification, and translocation. Moreover, regulatory genes are identified involved in regulating the related gene(s). Transferring and overexpressing of genes responsible for uptake, sequestering, translocation, and tolerance can be a successful approach to create some efficient plants capable of remediating HMs (Clemens et al. 2002). So the efficiency of HM phytoremediation depends directly on the processes such as metal uptake and translocation. Transferring the genes involved in any of these processes can enhance the efficiency of HM phytoremediation. Any combination of these genes can be overexpressed in transgenic plants. The overexpression of metal ligands and transporters, enzymes involved in the alteration of redox state of HM genes, can improve HM phytoremediation efficiency. Genetic engineering is capable of creating transgenic plants with different strategies to remediate HMs (Table 1). HMs can be accumulated in harvestable organs (phytoextraction) or absorbed to the root more than normal range (phytostabilization, rhizofiltration) by these plants. For instance, cysteine is the precursor molecule for the

Table 1 Transgenic plants with enhanced ability to bioremediate heavy metals

Plant	Transferred gene	Gene function	Heavy metal phytoremediated	References
Arabidopsis thaliana	Zinc finger transcription factor *AtZAT6*	Gene regulation	Cd	Chen et al. (2016)
Arabidopsis thaliana	*MAN3*	Encoding apoplastic endo-β-mannase	Cd	Chen et al. (2015)
Indian mustard (*Brassica juncea*)	*GS* and *γ-ECS*	Overproducing the enzymes gamma-glutamylcysteine synthetase (ECS) or glutathione synthetase (GS)	Cd and Zn	Bennett et al. (2003)
Indian mustard (*Brassica juncea*)	*γ-ECS*, *APS* or *GS*	Overexpression of adenosine triphosphate sulfurylase (APS), γ-glutamylcysteine synthetase (ECS), and glutathione synthetase (GS)	Se	Bañuelos et al. (2005), (2007)
Sterile line of poplar *Populus alba* X *P. tremula* var. glandulosa	*ScYCF1*	Encodes a transporter that sequesters toxic metal(loid)s into the vacuoles of budding yeast	Cd, Zn, Pb	Shim et al. (2013)
Arabidopsis thaliana	*ACBP1*	Overexpression of Arabidopsis acyl-CoA-binding protein binds Pb	Pb	Xiao et al. (2008)
Solanum lycopersicum	*Enterobacter cloacae ACC deaminase under* the control of CaMV 35S*, A. rhizogenes* RolD or tobacco pathogenesis-related PRB-1b promoters	Regulates stress-induced ethylene production	Several HMs	Grichko et al. (2000)
Brassica napus	*Enterobacter cloacae ACC deaminase*	Regulates stress-induced ethylene production	As (V)	Nie et al. (2002)
Petunia hybrida Vilm	*iaaM* and *ACC deaminase* genes	Plant senescence-inhibiting and growth-promoting regulation	Cu and Co	Zhang et al. (2008)
Indian mustard (Brassica juncea)	*AtAPS1*	Promotes selenate reduction as well as Se tolerance and accumulation	Se	Pilon-Smits et al. (1999)

synthesis of GSH, the predominant nonprotein thiol, which plays an important role in plant stress responses. GSH has been implicated in plant responses to toxic levels of HMs, as it is the precursor for the synthesis of PCs, the thiolate peptides involved in the detoxification of Cd and other HMs. Stimulation of the synthesis of building blocks for GSH may have been found to increase Cd tolerance in transgenic plants. *Arabidopsis thaliana* overexpressing cytosolic O-acetylserine(thiol)lyase gene (*OASTL*) showed Cd tolerance by increasing both cysteine and GSH availability (Domínguez-Solís et al. 2004).

6 Conclusion

The negative effect of HMs on organisms especially human being is one of the serious environmental concerns globally. HM bioremediation is a cost-effective and environmental friendly approach to decrease the entrance of HMs into the food chain. Phytoremediation is an effective bioremediation method. Therefore, molecular and cellular adaptation of plant cells in response to HM stress appears to be necessary to improve plant HM tolerance and detoxification. Identifying key pathways and components (enzymatic and nonenzymatic) of plants involved in the HM phytoremediation has paved the way of constructing efficient systems to bioremediate HMs from the soil, water, and air. Different HMs need different mechanisms to be bioremediated or tolerated. Moreover plants show completely different behaviors under excess amount of HMs in comparison with normal conditions. Many of these mechanisms have not been unraveled thoroughly and there is a need for extensive studies in this area. In addition, hypothesizing a common tolerance and bioremediation ability for all HMs is difficult. This requires experienced experts and more education in this field. The increasing identification and study of the remarkable natural variation in the capacity of plants to accumulate and tolerate HMs is continuing and will continue to provide a wealth of information. Therefore, concerted efforts by various research domains will further increase our understanding of the fundamental mechanisms involved in hyperaccumulation processes that naturally occur in metal-hyperaccumulating plants. This should allow us to develop plants that are more ideally suited for phytoremediation of HM-contaminated soils.

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Heavy Metal Stress-Responsive Phyto-miRNAs

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Contents

1 Introduction

In the twenty-first century, technological and industrial developments led to burgeoning population and extensive urbanization all around the world as a result. Excessive agricultural applications are utilized to meet the expectations of growing world population. Also, the need for natural sources in industry increased dramatically which escalated mining and metallurgy operations. All of these industries, urbanization, and agriculture factors contributed to atmospheric and environmental pollution collectively. This new era introduced a new kind of abiotic stress factor to plants which is named as "heavy metal stress" (HM stress). Heavy metals (HMs) are inorganic chemical compounds which have atomic mass greater than 20 µ and density over 5 $g.cm^{-3}$. HMs are inorganic compounds which are not biodegradable. Therefore, they accumulate in living organisms in time. HMs disturb various metabolic processes even in low concentrations and cause mutagenic effects as well as lead to cytotoxicity and genotoxicity. HMs are introduced to the environment by natural (geology, rock type, weather, volcanic activity), agricultural (organic and inorganic fertilizers, pesticides, liming, sewage sludge, irrigation water), industrial

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(mining, refinement, tailings, transport, smelting, and recycling of metals), and atmospheric sources (volcanic activity, airborne pollutants) as well as untreated wastewaters including elevated HMs (Nagajyoti et al. 2010; Emamverdian et al. 2015; Viehweger 2014).

Some HMs are essential for plant growth in trace levels. They either participate in redox reactions or take part in several enzymes. Cu, Zn, Fe, Mn, Ni, and Mo can be listed among the essential micronutrients in plants. Nevertheless, even the essential HMs present cytotoxic effects in plants. Nonessential HMs as Cd, Sb, Cr, Pb, As, Co, Ag, Se, and Hg and essential HMs in excess level have two different effect mechanisms in plants. HMs as Cr, Cu, Mn, and Fe are defined as redox-active metals which produce reactive oxygen species (ROS) by Haber-Weiss and Fenton reactions. ROS is a well-studied phenomenon in plants which is known to cause DNA damage, protein degradation, lipid peroxidation of cell membrane, disturbance in photosynthesis through degradation of photosynthetic pigments, loss of cell homeostasis, and cell death as a result. On the other hand, HMs as Cd, Ni, Hg, Zn, and Al are defined as non-redox-active metals. These group of metals cause damage in plants through depletion of glutathione, inhibition of antioxidant enzymes, induction of enzymes like NADPH oxidases which produce ROS, and binding to sulfhydryl groups of proteins (Emamverdian et al. 2015; Viehweger 2014; Lal 2010; Yadav 2010).

Plants adopt several adaptation mechanisms to cope with deleterious effects of heavy metal stress. As tolerance for all abiotic stress factors, the first step in heavy metal stress tolerance is perception of stress signals produced by the stress factor(s). For instance, copper signals calcium fluxes, cadmium and copper signal jasmonic acid, and cadmium and chromium signal MAPK pathways. All heavy metal stress signals facilitate signal transduction leading to biosynthesis of amino acids/organic acids, phytochelatins (PCs), metallothioneins (MTs), heat-shock proteins (HSPs), metal chelators, chaperons, ABC-type transporters, and CDF family metal transporters which are among the heavy metal binding or transporting mechanisms in plants (Viehweger 2014; Lal 2010; Clemens 2001).

In the past decade, microRNAs (miRNAs) were the topic of interest in plant abiotic and biotic stress responses. Also, transcription factors related to miRNA regulation are crucial in this mechanism (Fig. 1). MicroRNAs are an important part of gene regulation mechanism in eukaryotes. They are major members of small RNAs along with siRNAs (small interfering RNAs), piRNA (PIWI interacting RNA), and tsRNAs (transfer RNA-derived small RNAs). miRNAs are 20–35-nucleotide-long endogenous noncoding RNAs. They are well known to have negative, reversible, and sequence-specific regulatory roles in gene expression during plant–environment interactions, especially under abiotic stresses. There are miRNA genes (MIRs) responsible from primary miRNA (pri-miRNAs) transcription in plants which is mediated by RNA polymerase II. Even though the MIR expression is mainly regulated by cis- and trans-acting transcription factors on MIR promoter sites, epigenetic control of MIR regulation is also possible through acetylation of histone H3 by acetyltransferase. Pri-miRNAs which contain 5′ cap and 3′

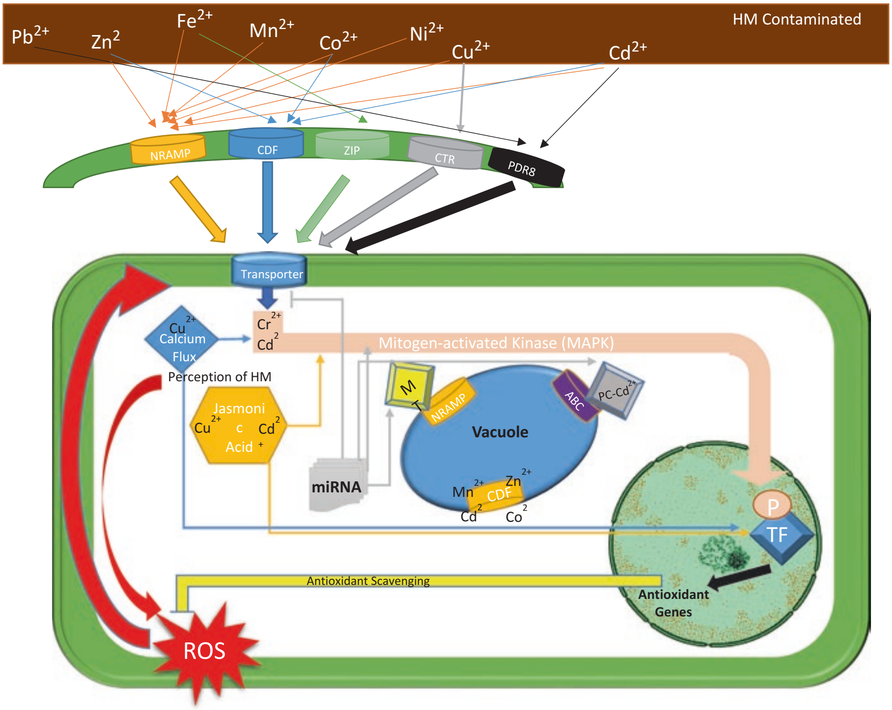

Fig. 1 Recognition of the heavy metal stress and main reponsive molecular pathways

poly-A tail are not in best fold-back shape and need processing into precursor miRNA in stem-loop structure. In plants, processing of pri-miRNAs is mediated by DICER-LIKE 1 (DCL1) with other RNA-binding proteins as HYL1. Due to the fact that plant MIRs contain introns, splicing is required as well as pri-miRNA processing. DCL1 and HYL1 also participate in splicing as well as processing of pri-miRNAs. After the pri-miRNA processing, miRNA duplex is methylated at 3′-end and loaded into ARGONAUTE protein (AGO). AGO protein is mainly decided according to the nucleotide placed at 5′. Loading of methylated miRNA duplex to AGO is known to require HEAT-SHOCK PROTEIN 90 (HSP90). In the last step, nuclear export signals (NES), which are dominant to nuclear localization signals, lead AGO to export to the cytoplasm (Gielen et al. 2012; Mendoza-Soto et al. 2012; Wang et al. 2019).

In plants, miRNAs cleave mRNA targets and generate 5′–3′ fragments. Uncapped 3′ fragments are targeted by exoribonucleases. Reduced mRNA level regulates translated protein products irreversibly. On the other hand, miRNAs can also match with complementary target mRNA sequence and block polyribosomes to bind on them. Therefore, repression of translation results in reduced protein accumulation reversibly. Other than these irreversible and reversible protein translation regulation mechanisms, recent studies emphasize miRNA-mediated DNA methylation as an alternative epigenetic regulation mechanism (Wang et al. 2019).

2 Heavy Metal Stress-Related Pathways via miRNA

miRNAs are reported to have roles in various abiotic stress responses as heat, cold, drought, and salinity nutrition deficiency on agricultural plants as cotton, soybean, rice, wheat, tobacco, etc. Plant miRNAs also regulate heavy metal response mechanisms as it is another abiotic stress factor. In this chapter, the miRNAs having a role in heavy metal stress response are discussed in the frame of their associated pathways. Tolerance to HMs can be evaluated under three main categories as chelation of HMs by metal-binding proteins as phytochelatins and metallothionines, regulation of metal uptake by transporter proteins as ABC-type transporters or CDF family transporters, and sequestration of HMs in vacuoles (Clemens 2001; Jalmi et al. 2018).

3 Phytochelatin- and Metallothionein-Related miRNAs

Metal-binding proteins are first introduced by Murasugi (1981) in *Schizosaccharomyces pombe* (Murasugi et al. 1981). They found Cd^{2+}-binding peptides named as cadystins. Today, their homologues are found almost in all plants and named as phytochelatins (PCs). PCs have important roles in obtaining homeostasis of metal ions in plant cells. Phytochelatins consist of δ-glutamate–cysteine repeats ranging between 2 and 11. The last amino acid binding to this chain is varied among species which can be alanine, serine, or glutamine. PCs are not synthesized in translational process. They are produced by reduction of glutathione (GSH) mediated by GSH synthetase enzyme under the control of GSH2 gene. δ-glutamate–cysteine chains are synthesized by PC synthase under the control of CAD1, PCS1, and PCS2 genes. The main inducer in PC synthesis is Cd^{2+} (Joshi et al. 2015). Nevertheless, other HMs as Cu^{2+} and Ag^{2+} can also induce synthesis. CAD1 was first identified in Cd-sensitive Arabidopsis mutant by Howden et al. (1995) and defined as a structural gene for PC synthesis. Bai et al. (2019) presented that cloning of three duplicated BnPCS genes (BnPCS-A, BnPCS-B, and BnPCS-C) from *Brassica napus* to PCS1-deficient CAD1–3 mutant *Arabidopsis thaliana*, which is susceptible to Cd, results in overexpression of PCS1 and Cd tolerance under the cascade of Cd treatments. Talebi et al. (2019) evaluated Azolla species for their potential in phytoremediation of HMs as Cu, Zn, Ni, and Cd. They highlighted that manipulation of PCS1 gene has the potential in phytoremediation as PCs tightly bind HMs in complex form and sequestrate them in vacuoles. They also utilize the essential metal ions as Cu^{2+} or Zn^{2+} into apoenzymes for their catalytic activities, or into the nucleic acid structures as zinc fingers. Yamazaki et al. (2018) reported that recombinant OsPCS1 and OsPCS2 proteins presented different metal activation. In their study, OsPCS1 was strongly activated by As(III) despite the fact that Cd is the main activator of PCS1 in many plants. On the other hand, Cd activated OsPCS2 stronger than As(III) in rice.

They also suggested that OsPCS2 is a major isozyme controlling PC synthesis and the PCs are important for As tolerance in rice, based on genetically engineered plant studies having their OsPCS2 expression silenced via RNA interference (OsPCS2 RNAi). Recent studies presented that HMs as Ag and Fe modulate transcription of genes as glutathione synthetase (GS2), which mediates synthesis of δ-Glu-Cys-Gly substrate for PC synthase, glutathione reductase (GR), and phytochelatin synthase (PCS1) genes even in nanoparticle size. The whole-genome cDNA expression microarrays of Ag nanoparticle-exposed Arabidopsis plants presented 286 upregulated and 81 downregulating genes including heavy metal- and oxidative stress-related genes (SJ-BS for AEI in 2018). Several miRNAs are defined to regulate transcription levels of PCs against HMs. Predicted miRNAs targeting PCs were investigated in psRNATarget database. A total of 53 miRNAs were identified which were belonging to 8 different plant species (*S. lycopersicum*: 1; *Z. mays*: 1; *P. trichocarpa*: 4; *B. distachyon*: 5; *O. sativa*: 7; *A. thaliana*: 8; *S. bicolor*: 9; *M. truncatula*: 18). Twenty-nine of these putative miRNAs were found to utilize cleavage inhibition type for target mRNA, while 24 putative miRNAs utilize repression of translation (Filiz et al. 2019). miRNA395 is also induced by Cd^{2+} and it regulates sulfate assimilation pathway. Induction of PCs consumes sulfate in the synthesis of polypeptide chains. miRNA395 is proposed to have indirect regulation effect by mediating sulfate insufficiency (Gielen et al. 2012). *Platanus acerifolia* plants are also investigated under Pb stress. The novel pla-mir3, pla-mir59, pla-mir108-3p, and pla-mir45-3p miRNAs are identified and suggested to have roles in PC biosynthesis and ROS scavenging. pla-mir3 targets glutaredoxin gene while pla-mir108-3p and pla-mir45-3p target glutathione S-transferase gene. Glutathione S-transferase mediates binding of glutathione to heavy metals as well as having peroxidase activity during ROS scavenging. On the other hand, glutaredoxins are oxidoreductases which perceive redox potential of glutathione and mediate reversible electron transfer between thiol groups of target proteins and glutathione redox buffer. Moreover, downregulation of pla-mir108-3p, pla-mir3, and pla-mir59 in XLM-12-40 sRNA library compared with their expressions in XLM-0 library and upregulation of glutathione S-transferase and glutaredoxin XLM-12-40 library was confirmed by qRT-PCR analysis (Wang et al. 2009). Small RNA libraries for radish (*Raphanus sativus*) under Cd stress are constructed (Xu et al. 2013). Differentially expressed miRNAs and their targets are predicted by using degradome analysis. Fifteen known and 8 novel miRNA families were identified. Among these miRNAs, rsa-miR393 is found targeting phytochelatin synthase 1 (PCS1)-encoding transcript under Cd stress which suggests regulation of miRNAs in reduction of HM mobility and sequestration of phytochelatin–metal complexes into the vacuole.

Another major group of metal-binding proteins is metallothioneins (MTs). They are low-molecular-weight metal-binding proteins that are rich in cysteine content and are found in many different eukaryotic organisms as well as prokaryotes. Unlike phytochelatins, which are peptide products synthesized enzymatically (PC synthases), MTs are synthesized by mRNA translation (Cobbett 2000; Ernst et al. 1992). Many internal and external factors such as seed and root development (Yuan et al. 2008), osmotic stress, drought, nutritional deficiency, natural senescence, wounding, viral infection, and hormone secretion are effective in the expression of MTs (Yang and Chu 2011). MTs in plants have a wide variety of functions in other organisms due to their different molecular characteristics and structural differences. The expression of plant MTs is tissue specific and under developmental control, and some key plant hormones play an important role in regulating the expression of these genes (Leszczyszyn et al. 2013). Metallothioneins show affinity for a large number of metals including copper, zinc, cadmium, and arsenic. These ligands, which play an important role in eliminating the toxicity of heavy metals by cellular sequestration, also play a role in different mechanisms such as homeostasis of intercellular metal ions and regulation of metal transport (Guo et al. 2013). In addition to their role in heavy metal detoxification, MTs are involved in ROS scavenging (Yamauchi et al. 2017; Zhu et al. 2010), repair of plasma membranes, cellular proliferation, maintenance of redox levels (Bell and Vallee 2009), and repair of damaged DNA (Emamverdian et al. 2015). MTs in plants are divided into four subgroups according to the arrangement of cysteine residues (Cobbett and Goldsbrough 2002). Type 1 MTs generally express the roots. type 2 MTs are mostly found in leaves, while type 3 MTs are abundant in ripe fruits. Expression of type 4 MT occurs in developing seeds. Almost all MT varieties and isoforms described in plants are attached to heavy metals or serve as metal chelators or storage room (Emamverdian et al. 2015).

Cysteine is one of the precursor molecules for different S-rich cellular components as metallothioneins, phytochelatins, glutathione, etc. miR395 is known to regulate S transporters for S equilibrium. According to Mitra (2018), miR395 targets the APS genes which encode ATP sulfurylase isoforms and also low-affinity sulfate transporters as Sultr2;1 located in the xylem parenchyma cells of roots and shoots. miR395-overexpressing plants distribute S from older leaves to the youngers. SLIM1 which is a TF in S-assimilation pathway controls the induction of miR395. Therefore, it has indirect effects on metallothionine biosynthesis under different stress factors (Shahzad et al. 2018). Likewise, recent deep sequencing study aimed to identify and characterize miRNAs and their potential targets in *Nicotiana benthamiana* under phosphate starvation. miR398 is found targeting three transcripts. Cu/Zn superoxide dismutase (CSD) is known as conserved target for miR398. However, metallothionein is a novel target for miR398 as well as blue copper-binding protein (BCBP) (Huen et al. 2018). In *Brassica napus*, long noncoding RNAs (lncRNAs) are also investigated under Cd stress. LncRNAs have base-pair coupling with target mimics or miRNAs. Target mimics are small noncoding RNAs which compete with miRNAs endogenously. LncRNAs protect miRNAs from this mimicry. Thirty-six potential endogenous target mimics (eTMs) were identified for 11 Arabidopsis miRNAs while 189 eMTs were identified for 19 rice miRNAs in genome-wide profiling studies. LncRNAs and miRNAs are investigated in rapeseed plants under Cd stress and some lncRNAs were identified for some miRNA targets which were related to Cd uptake, transportation, and detoxification. For an instance, TC201601 encodes metallothionein protein and it is regulated by miRNA2678.

Also, EL628609 regulated by m0079_3p, TC203372 regulated by miRNA1533, and ES967796 regulated by miR4424 encode metal transporter NRAMP3, Cu/Zn-superoxide dismutase copper chaperone precursor and ABC transporter, and permease protein, respectively, under Cd stress (Feng et al. 2016). Artificial miRNA approach is another useful tool for confirming miRNA targets. Expression of the MT-1-4b gene, which is responsible from metal-binding metallothionein encoding, is targeted in rice. Two 21-nucleotide amiRNA sequences were designed by the customized version of the Web MicroRNA Designer platform (http://wmd.weigel-word.org) and replaced the sequence of endogenous microRNA, osa-MIR528. Transgenic rice plants presented defective anther development and pollen formation due to elevated ROS levels in the absence of osa-MIR528-regulated MTs (Hu et al. 2011). Metallothionein-like protein 3 is known to have roles in different abiotic stresses other than HM stress as hormone treatment, heat shock, cold, drought, salinity, wounds, and pathogen infection (Mekawy et al. 2018). miR1131 is reported to be a putative target for metallothionein-like protein 3 regulation in drought-stressed wheat plants (Kantar 2015).

4 Transporter Family-Related miRNAs

Various plasma membrane transporters from many different families have been reported to be involved in the uptake of HMs and homeostasis. ATP-binding cassette (ABC) transporters, natural resistance-associated macrophage protein (NRAMP), cation diffusion facilitator (CDF), ZIP family transcription factors, copper transporters (CTR), heavy metal ATPases (HMAs), and oligopeptide transporters (OPT) play a fundamental role in the response to metals and contribute to the detoxification of heavy metals as part of the metal transport, chelation, and sequestration network (Yu et al. 2017).

Plant vacuole is the major storage compartment for excess heavy metals to balance the cytosolic metal concentration (Ernst et al. 1992). Cation diffusion facilitator (CDF) transporters located in vacuole membrane are important proteins that mediate the transport of metal cations such as Zn^{2+}, Cd^{2+}, Co^{2+}, Ni^{2+}, and Mn^{2+} from the cytoplasm to the vacuole (Yang and Chu 2011). On the other hand, the plant natural resistance-associated macrophage protein (NRAMP) transporter family plays an important role in transporting divalent metal cations from vacuole to cytoplasm when they are required in metabolic processes (Lanquar et al. 2010; Oomen et al. 2009; Wei et al. 2009). In addition, vacuolar membrane-localized ATP-binding cassette (ABC) transporters which play a crucial role in transporting phytochelatin-heavy metal complexes to the vacuole and tonoplast-localized cation/proton exchanger (CAX) transporters that carry the heavy metals to the vacuole by direct proton exchange are proteins that play major roles in sequestration of heavy metals (Bovet et al. 2005; Koren'kov et al. 2007). Numerous studies were conducted to indicate that transporter proteins are regulated by various miRNAs under heavy metal stress. Conserved and non-conserved miRNAs were identified by high-throughput sequencing in *B. napus* seedlings exposed to heavy metal stress. Eight hundred two target genes were identified from three different categories for the 7 miRNA families (Zhou et al. 2012). Many of these target genes encode transcription factors and proteins involved in the developmental processes and hormone-dependent pathways. Within these target genes, ABC and NRAMP transporters have been identified for miR159 and miR167, respectively. In addition, miRNA target genes encode critical enzymes and proteins for HM tolerance. miR156 provides regulation of the glutathione-γ-glutamylcysteinyl transferase (GGT) enzyme, which is the component of one of the main mechanisms for detoxification of heavy metals in *B. napus* cell together with phytochelatin synthase (Zhou et al. 2012). Another HM transport protein family is found to be a target for two different miRNAs. nta-miRNA6149a and nta-miRNA6149b were reported to contribute to improving Cd tolerance in tobacco through CTR2 (Cu transporters) (He et al. 2016). Hauqe et al. (2016) showed that col-miR159 and col-miR167 were involved in heavy metal stress response to As, Mn, and Cr in jute. It was reported that col-miR159 suppressed the expression of the ABC transporter gene, while col-miR167 suppressed the expression of the ARF8 gene.

Heavy metal ATPases (HMAs), known as the P1B-type ATPases, are one of the protein families that participate in metal transfer. The P1B subfamily of ATPases is divided into two subgroups, known as mono- and divalent metal transporters. Recent studies have shown that HMAs in the vacuolar membrane are involved in vacuole transport of Zn and Cd. Plants overexpressing the HMA3 gene have been reported to have increased tolerance to Cd (Morel et al. 2009). Moreover, recent studies have shown that miRNAs can regulate HMA and miRNAs-HMA expression network participation in the response to heavy metal stress. These findings showed that miRNAs play an important role through regulation of HMA target genes such as TaHMA2;1 in Cd-stressed wheat (*Triticum aestivum* L). Moreover, it has been reported that miRNA-2B36279 regulates the expression of TaHMA2;1, TaHMA2;2, TaHMA2;4, TaHMA2;5, and TaHMA2;6, while miRNA-4B11876, miRNA4B3407, miRNA-4B16562, and miRNA-4B13629 regulate TaHMA3;1 while miRNA-2B_28883 regulates TaHMA1. Results showed that microRNA-2B_36279∗ can regulate TaHMA2;1, TaHMA2;2, TaHMA2;4, TaHMA2;5, and TaHMA2;6, while microRNA-4B_11876, microRNA4B_3407, microRNA-4B_16562, microRNA-4B_13629, and microRNA-2B_40139∗ can regulate TaHMA3;1 and microRNA-2B_28883 can regulate TaHMA1; 4 (Zhou et al. 2019).

As another transporter family, ZIP family membrane transporters also play important roles in the transport of four main micronutrient/heavy metals, especially Zn, Fe, Mn, and Cu, and in balancing metal uptake and homeostasis (Milner et al. 2013). ZIP proteins have eight potential transmembrane domains. They have a variable domain between TM-3 and TM-4, where the amino- and carboxyl-terminals are located on the outer surface of the plasma membranes. This variable region involves a potential metal-binding domain rich in conserved histidine residues (Guerinot 2000). One hundred forty-one known miRNAs from 48 families and 39 known miRNAs from 23 families were identified in the shoots and roots of *Brassica napus* plant under Cd stress, respectively (Tang et al. 2014). In addition, 448 genes in the shoot and 1044 genes in the root were upregulated, while 645 genes in shoots and 572 genes in roots were downregulated. The analysis showed that these genes encode the metabolite synthase, signal molecules, and ABC transporters in roots, and encode ribosomal protein and carotenoid biosynthesis in shoots. Six novel and ten known mRNA-miRNA association candidates showed opposite expression profiles. These different proteins, which are involved in stress responses, can use a common mechanism in different stress situations. Zhang et al. (2013) showed that miR395 plays an important role in the detoxification of Cd and that the target gene SULTR2;1 (sulfate transporter) is also transcriptionally regulated by the same miRNA in sulfate deficiency. At the physiological level, Cd-induced production of sulfate-containing compounds (e.g., glutathione, GSH; phytochelatins, PCs; metallothioneins, MTs) and sulfate uptake can be correlated (Huang et al. 2010). Under cadmium toxicity, 19 specific miRNAs and 34 miRNA target genes, some of which were involved in the regulation of heavy metal-induced stress response, were identified. These target genes include some proteins associated with plant metabolism such as WAK-like protein, GHMP kinase, ABA/WD-induced proteins, F-box protein, LRR domain-containing protein, ATP-dependent protease, and hydrolase. The targets of Cd-responsive miRNAs include proteins such as DCL1, OsDCL1, and ARGONAUTE, which are involved in miRNA processing. Lima et al. (2011) determined 46 miRNA families under aluminum stress. In *Oryza sativa* spp. japonica cv. Nipponbare cultivar, 13 miRNAs were downregulated while 6 miRNAs were upregulated. In *Oryza sativa* spp. indica cv. Embrapa Taim cultivar, 5 miRNAs were downregulated in the root and 3 miRNAs were upregulated. Putative targets of Al-upregulated miRNAs include those related to the genes such as ZIP protein (miRNA166k, 1), ABC protein (miRNA819), auxin response factor (miRNA160e), superoxide dismutase, copper ion-binding protein (miRNA528), and phosphate transporter (miRNA399d), while the putative targets of downregulated miRNAs target genes as inhibitory response protein (miRNA393b), superoxide dismutase 2 (miRNA398a, b), sulfate transporter 3, and sulfate transporter 2.1 (miRNA395a). As a result of analysis of arsenide-stress-responsive miRNAs, 67 miRNAs were identified in *Oryza sativa* L. indica roots. Potential targets of these miRNAs have been reported as transcription factors, protein kinases, and DNA- or metal ion-binding proteins (Liu and Zhang 2012).

5 Heat-Shock Protein-Related miRNAs

Plants inherently respond to stress by triggering activation of genes called stress genes. As part of this response, a number of genes are induced to synthesize a group of proteins named as heat-shock proteins (HSPs) (Gupta et al. 2010). Stress-induced

HSPs play a crucial role in the protection of cellular homeostasis by helping to correct the folding of nascent and misfolded proteins due to stress, in the prevention of protein aggregation, or in the selective degradation of misfolded or denatured proteins (Park and Seo 2015). HSPs function as molecular chaperones. HSPs with molecular weights ranging in 10–200 kDa are characterized as chaperones involved in inducing signals under stress conditions (Schöffl et al. 1999). Heat-shock proteins induced in response to heavy metal stress in plants are divided into five subgroups: HSPs70, HSPs60, HSPs90, HSPs100, and sHSPs (Hasan et al. 2017). Transcriptomic and proteomic analyses in many plant species indicate that HSPs, such as HSP90, HSP70, and HSP60, express under various metal stresses, retain the accumulation of newly synthesized proteins, and ensure correct folding during the transfer to the destination and play a significant role in the sequestration and detoxification of metal ions (Haap et al. 2016; Rodríguez-Celma et al. 2010; Pratt and Toft 2003). Some of the genes regulated by miRNAs induced by heavy metal stress in plants are closely related to plant metabolic processes. Valdés-López et al. (2010) demonstrated the effect of manganese toxicity on miRNA expression profile in *Phaseolus vulgaris*. Under manganese toxicity, 11 miRNAs were strongly induced in nodules, while the other 11 miRNAs were strongly inhibited in leaves and roots. Target genes of these miRNAs include heat-shock proteins, receptor kinase proteins, LRR resistance-like proteins, transport inhibitor response proteins, and GTP-binding proteins.

Nanoparticles are an important part of environmental pollutants that have emerged in the last decade. Nanoparticles are used extensively in the industrial field to produce light and strong materials, such as paint and cosmetic industry products. In this context, it is important to identify the effects of various nanoparticles such as aluminum oxide nanoparticles on the growth and development of agricultural plants and the potential miRNAs that play an active role in this process. A significant increase in the expression of miR395, miR397, miR398, and miR399 in the Al_2O_3-treated *Nicotiana tabacum* compared to the control plant showed that these miRNAs were effective in responding to nanoparticle stress (Burklew et al. 2012). In particular, mir399 and mir395 play important roles in stress response to aluminum oxide nanoparticles, apart from their known roles in phosphate transporter and sulfate transporter target gene regulation and phosphate and sulfate regulation, respectively. Expression of 69 miRNAs from 18 families in *Brassica juncea* induced by arsenic stress has been shown to vary, and putative target genes of miRNAs include various developmental processes as sulfur uptake, transport, and assimilation (miRNA838, miRNA854) and genes related to hormonal signaling pathways. He et al. (2016) identified 28 known and 5 specific miRNAs with different expression profiles associated with Cd tolerance in their extensive miRNA analysis in control- and Cd-treated tobacco plants. Some of these miRNAs have been reported to be associated with Cd tolerance by acting in cell growth, ion homeostasis, stress defense, and antioxidant and hormone signaling.

6 Transcription Factor-Related miRNAs

There is a wide variety of transcription factors in eukaryotes; hence 3–10% of eukaryote genes encode TFs. TFs can regulate gene expression as activators or repressors depending on their variable sites located neighboring the DNA-binding sites. Interaction of TFs with other proteins is crucial in their regulation function. According to their DNA-binding domains, TFs can be categorized under five superclasses numbered as 1, 2, 3, 4, and 0. They are named as basic domains, zinc-coordinating DNA-binding domains, helix–turn–helix, beta-scaffold factors with minor groove contacts, and other TFs, respectively. Many TF families are also common among other eukaryotes due to their common ancestry. Therefore, TFs can be further classified as plant-specific TFs (AP2/ERF family, WRKY family, NAC family, TCP family), TFs conserved in eukaryotes (bZIP family, bHLH family, MYB family, HSF family), and TFs without DNA-binding site (B-Box zinc finger family, Aux/IAA family, JAZ protein family) in plants (Gonzalez 2015; Hong 2015).

miRNAs play a regulatory role in downstream of TFs as they target mRNA expression posttranscriptionally, while TFs regulate mRNA transcription. TFs mediate miRNA expression as miRNAs can repress TF expression in a feedback-loop (FBL) and feedforward-loop (FFL) manner. In FBL interaction of TFs and miRNAs, TFs regulate a miRNA target and this particular miRNA represses that TF in return. In FFL mechanism, TFs regulate expression of a target gene directly by binding DNA-binding site and indirectly through miRNAs. These miRNAs regulate related target gene afterwards (Mullany et al. 2018). Even though the topic of this chapter is HM-related phyto-miRNAs, it is not feasible to evaluate HM-related miRNAs separated from TFs due to their intertwined regulatory mechanisms. In this context, we summarized TFs which are targeted by HM-related phyto-miRNAs in Table 1.

7 Future Prospects

The heavy metal stress-responsive pathways are so important for plants to overcome the stress they have subjected to as a result of industrialization. In recent years, the scientific improvements in sequencing technologies have caused identification of new miRNAs. Unfortunately, the important part is to identify the relations between phyto-miRNAs and their effective pathways. In this circumstance, the indicator plants are becoming important to investigate on.

Table 1 TFs which are targeted by HM-related phyto-miRNAs

Metal	miRNAs	Targets	Classification	References
Al, Mn	miRNA172	ARF transcription factors, Apetala-2 like TFs	TFs without DNA-binding sites, plant-specific TFs	Valdés-López et al. (2010); Lima et al. (2011)
Mn	miRNA167	ARF transcription factors	TFs without DNA-binding sites	Zhou et al. (2012); Valdés-López et al. (2010)
Mn	miRNA170	SCL transcription factors	TFs conserved in eukaryotes	Valdés-López et al. (2010)
As, Cd	miRNA159	MYB, TCP transcription factors	Plant-specific TFs, TFs conserved in eukaryotes	Xu et al. (2013); Zhou et al. (2012); Srivastava et al. (2013); Chen et al. (2012)
As	miRNA164	NAC transcription factors	Plant-specific TFs	Srivastava et al. (2013)
As	miRNA165	HD-ZIP transcription factors	TFs conserved in eukaryotes	Srivastava et al. (2013)
As	miRNA169	NFYA transcription factors	TFs conserved in eukaryotes	Liu and Zhang (2012)
As	miRNA172	AP-2, TOE2, TOE1, SNZ, and SMZ transcription factors	Plant-specific TFs, TFs conserved in eukaryotes	Srivastava et al. (2013)
As	miRNA319	Myb (Myb33/65/104) and TCP (TCP4/10) transcription factors	Plant-specific TFs, TFs conserved in eukaryotes	Srivastava et al. (2013)
As	miRNA390	TAS3-ARF transcription factors	TFs without DNA-binding sites	Srivastava et al. (2013)
Hg	miRNA164	NAC transcription factors	Plant-specific TFs	Zhou et al. (2012); Zeng et al. (2012)
Hg	miRNA171	SCL transcription factors	TFs conserved in eukaryotes	Yu et al. (2017); Ding et al. (2011)
Hg	miRNA393	bHLH transcription factors, transport inhibitor response 1 TFs	TFs conserved in eukaryotes	Lima et al. (2011)
Cd	miRNA394	F-box TF	TFs conserved in eukaryotes	Huang et al. (2010); Sun (2012)
Cd	miRNA529	Apetala-2-like TFs, squamosa promoter-binding protein-like TFs	Plant-specific TFs	Zhou et al. (2008)
Cd, Hg, Al	miRNA 319	TCP transcription factors	Plant-specific TFs	Zhou et al. (2008); Chen et al. (2012)

(continued)

Table 1 (continued)

Metal	miRNAs	Targets	Classification	References
Cd, Hg, Al	miRNA171	SCL transcription factors	TFs conserved in eukaryotes	Zhou et al. (2012); Zhou et al. (2008); Xie et al. (2007)
Cd	miRNA156	SBP transcription factors	Plant-specific TFs	Xu et al. (2013); Ding et al. (2011); Xie et al. (2007)
Cr	miRNA156	SBP transcription factors	Plant-specific TFs	Bukhari et al. (2015)
Cr, Al	miRNA169	Nuclear transcription factors	–	Lima et al. (2011); Bukhari et al. (2015)
Cd, Hg, Al	miRNA393	bHLH transcription factors	TFs conserved in eukaryotes	Zhou et al. (2008); Xie et al. (2007)
Hg, Mn	miRNA172	AP2 transcription factors	Plant-specific TFs	Zhou et al. (2012); Valdés-López et al. (2010)
Cd, Hg	miRNA164	NAC, CUP transcription factors	Plant-specific TFs	Zhou et al. (2012); Huang et al. (2009)
Cd	miRNA96	Growth-regulating transcription factors	–	Ding et al. (2011)
Cd	miRNA444b.1	MADS-box transcription factors	–	Ding et al. (2011)
Cd, Al, Mn, As	miRNA156	SBP transcription factors	Plant-specific TFs	Yu et al. (2017); Zhou et al. (2012); Huang et al. (2010); Lima et al. (2011); Zeng et al. (2012); Srivastava et al. (2013); Ding et al. (2011); Xie et al. (2007)
Cd	miRNA157	Squamosa promoter-binding protein (TF)	Plant-specific TFs	Xu et al. (2013)
Cd, Al, Al2O3 nanoparticles, As	miRNA159	MYB and TCP transcription factors	Plant-specific TFs; TFs conserved in eukaryotes	Zhou et al. (2012); Burklew et al. (2012); Zeng et al. (2012); Srivastava et al. (2013); Chen et al. (2012); Huang et al. (2009)
Cd, Al, Mn	miRNA160	Auxin-responsive factors (ARFs) (TF)	TFs without DNA-binding sites	Huang et al. (2009, 2010); Valdés-López et al. (2010); Lima et al. (2011); Chen et al. (2012)

(continued)

Table 1 (continued)

Metal	miRNAs	Targets	Classification	References
Cd, Hg, Al, As	miRNA164	NAC (TF)	Plant-specific TFs	Zhou et al. (2012); Huang et al. (2009, 2010); Zeng et al. (2012); Srivastava et al. (2013)
Cd, Al, As	miRNA166	HD-ZIP (TF)	Plant-specific TFs	Xu et al. (2013); Yu et al. (2017); Lima et al. (2011); Zhou et al. (2008); Ding et al. (2011)
Cd, Hg, Mn, Al_2O_3 nanoparticles, As	miRNA167	Auxin-responsive factors (TF)	TFs without DNA-binding sites	Zhou et al. (2012); Huang et al. (2009, 2010); Valdés-López et al. (2010); Burklew et al. (2012); Srivastava et al. (2013)
Mn	miRNA170	Scarecrow-like (SCL) (TF)	TFs conserved in eukaryotes	Valdés-López et al. (2010)
Hg, Mn, Al	miRNA172	Apetala-2-like (TF)	Plant-specific TFs	Valdés-López et al. (2010); Lima et al. (2011)
Cd, Hg, Al, Mn, As	miRNA319	TCP (TF)	Plant-specific TFs	Valdés-López et al. (2010); Srivastava et al. (2013); Zhou et al. (2008); Chen et al. (2012)
Cd, Hg, Al	miRNA393	bHLH (TF), transport inhibitor response	TFs conserved in eukaryotes	Huang et al. (2009, 2010); Lima et al. (2011); Zhou et al. (2008); Xie et al. (2007)
Cd, Hg, Al, Al_2O_3 nanoparticles, As	miRNA396	50S ribosomal protein L20, growth-regulating factor, auxin response factor 8 (ARF8) (TF)	TFs without DNA-binding sites	Xu et al. (2013); Yu et al. (2017); Zhou et al. (2012); Valdés-López et al. (2010); Zeng et al. (2012); Chen et al. (2012)
Cd	miRNA529	Apetala-2-like (TF), squamosa promoter-binding protein-like (TF)	Plant-specific TFs	Zhou et al. (2008, 2012)
Mn	miRNA1509	bHLH104	TFs conserved in eukaryotes	Valdés-López et al. (2010)
Cd	miRNA398	GATA-type zinc finger transcription factor	TFs without DNA-binding sites	Fang et al. (2013)

(continued)

Table 1 (continued)

Metal	miRNAs	Targets	Classification	References
Cd	miRNA319	PCF transcription factor 2	–	Fang et al. (2013)
Pb	miRNA172	MYB68, AP2 TFs	TFs conserved in eukaryotes, plant-specific TFs	He et al. (2014)
Pb	miRNA396	Growth-regulating factor TFs	–	He et al. (2014)
Pb	miRNA159	TCP TFs	Plant-specific TFs	He et al. (2014)
Pb	miRNA164	CUC TFs	Plant-specific TFs	He et al. (2014)
Pb	miRNA414, miRNA833a, miRNA396	RAX1, RAX 2, RAX 3	–	He et al. (2014)
Pb, Cr	miRNA156	Squamosa promoter-binding protein	Plant-specific TFs	Liu et al. (2015); He et al. (2014)
Cr	miRNA164	NAC1, ethylene-responsive transcription factor	Plant-specific TFs	Liu et al. (2015)
Cr	miRNA172	IIIA transcription factor	–	Liu et al. (2015)
Cr	miRNA415	Ethylene-responsive transcription factor	–	Liu et al. (2015)
Cr	miRNA5293	TCP15, TCP21, TCP6 transcription factor	Plant-specific TFs	Liu et al. (2015)
Cd, Al	miRNA169	AP2 domain-containing, CCAAT-binding transcription factor	Plant-specific TFs	Xu et al. (2013); Zeng et al. (2012)
Cd	miRNA408	Auxin response factor 6(ARF6)	TFs without DNA-binding sites	Xu et al. (2013)
Al	miRNA528	Fe-box	–	Lima et al. (2011)
Al	miRNA1561	Squamosa promoter-binding protein	Plant-specific TFs	Lima et al. (2011)

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Heavy Metal Phytotoxicity: DNA Damage

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Abbreviations

AP	Apurinic/apyrimidinic
APE	Apurinic/apyrimidinic endonuclease
DSB	Double-stranded breaks
EMS	Ethyl methane sulfonate
FISH	Fluorescent in situ hybridization
m5C	5-Methylcytosine
MMS	Methyl methane sulfonate
MNC	Micronucleus test
RAPD	Random amplified polymorphic DNA
SCE	Sister chromatid exchange
SSB	Single-stranded breaks

1 Introduction

Heavy metals are considered as significant environmental pollutants. Heavy metals are referred to as metallic elements that have relatively high density and toxicity even at very low concentration (Nagajyoti et al. 2010). Heavy metals are the group of metals and metalloids having genotoxic effects with atomic density greater than 4 g/cm^3 (Hawkes 1997). Heavy metal stress results in the generation of reactive oxygen species (ROS), which are relatively very toxic, compared to molecular oxygen (Ercal et al. 2001).

Heavy metals induce several toxic effects on cellular and molecular levels. It can damage various cellular components such as enzymes, proteins, membranes, and DNA. Nowadays, advances in molecular biology have led to the development of several DNA damage detection techniques. These DNA damage detection techniques involve different plant-related assays such as Allium assay, comet assay, MNC, TUNEL, and DNA-based techniques, like molecular markers (RAPD). DNA-based techniques are used to evaluate the variation at the DNA level, and differences can clearly be shown when comparing DNA fingerprints of control plant with stressed one (Enan 2006).

Like all living organisms, plants are also sensitive to the deficiency as well as excess of micronutrients. Some heavy metals are essential for plant growth up to a certain concentration; beyond that all micronutrients become poisonous and start hindering plant metabolic activities (Prasad 2004). Nowadays, agricultural soils get more and more contaminated by heavy metals and it becomes a critical environmental concern due to their potential adverse ecological effects. Toxic heavy metals in soil are considered as soil pollutants due to their widespread occurrence and their toxic effects on plant growth (Sharma and Dubey 2005).

Heavy metals cause severe damage to the genetic material of the plants. Heavy metals get bound to the cell nucleus and cause promutagenic damage. DNA damage includes base modifications, DNA strand breaks (SSBs and DSBs), DNA-protein cross-links, rearrangement, depurination, hypomethylation, etc. (Benavides et al. 2005). The molecular and cellular mechanism of metal genotoxicity in plants is still unknown, even though the toxic effects on crop production have long been recognized (Bertin and Averbeck 2006).

2 Heavy Metals

2.1 *Lead (Pb)*

Lead (Pb) is one of the most useful and it is also one of the most toxic metals. Several literatures show the different physiological, morphological, and biochemical changes in plants due to Pb stress (Burzyński and Kłobus 2004).

Pb inhibits the growth of plant, seed germination, its development, cell division, transpiration, root elongation, chlorophyll production, lamellar organization, etc. (Liu et al. 2008). However, very less data are available on the genotoxic effects of Pb on plant (Pourrut et al. 2011). In 2004 a group of researchers showed the effect of Pb on lupin roots; it causes SSBs (Rucińska et al. 2004). The other study conducted their research on *Vicia faba*. They used different concentration of Pb on *Vicia faba* plant and found that Pb shortened the mitotic stage and prolonged the interphase resulting in DNA strand breaks and prolonged the interphase of cell cycle at high concentration (Shahid et al. 2011).

The action mechanism of Pb genotoxicity is complex and not yet well understood. In vitro studies demonstrated that lead interacts with DNA or some associated proteins. Pb reacts with sulfhydryl groups and the phosphate backbone of DNA molecule (Tajmir-Riahi et al. 1993). Pb decreases the effectiveness of DNA polymerase enzyme which leads to the disturbance in DNA and RNA synthesis (Hartwig et al. 1990).

2.2 *Arsenic*

Arsenic (As), a naturally occurring element widely distributed in the earth’s crust, is one of the most toxic pollutants in the environment (Yi et al. 2007). As uptake by plants is in two different forms, arsenite and arsenate, which is greatly influenced by soil texture and competing phosphates. Arsenic was displaced by low levels of phosphates from soil particles to increase uptake by plants (Peterson et al. 1981). Phosphorus takes place in several cell reactions and due to having same chemical structure of phosphorus and arsenic, phosphorus is readily replaced by arsenic in the phosphate groups of DNA. Both sodium arsenate and sodium arsenite increased the chromosomal aberrations in *Allium cepa* (Wu et al. 2010).

As mainly reacts with thiol and sulfhydryl groups of enzymes required for the synthesis of genetic material of plant (Zhao et al. 2010). Enzyme inhibition (DNA repair enzymes) is the major cause of DNA damage in plants by As (Patra et al.

2004). It lies in the overproduction of ROS which leads to oxidative DNA damage (Lin et al. 2007). DNA damages include DNA lesions (sugar and base), SSBs, DSBs, abasic site, DNA-protein cross-links, etc. (Roldán-Arjona and Ariza 2009). Other than direct oxidation, As indirectly damages DNA by generating ROS, which attack lipids (Mancini et al. 2006).

2.3 Mercury

Mercury (Hg) has gained special attention due to its high toxicity and widespread occurrence (Regier et al. 2013). Hg was found predominantly in Hg^{2+} form in soil and water. Hg^{2+} is highly toxic and water soluble, and can readily accumulate in higher plants (Elbaz et al. 2010). Hg is highly reactive, and reacts with the sulfhydryl groups of different enzymes responsible for maintaining the structure and function of cell (Zhou et al. 2007).

Hg is reported to disrupt the integrity of cell membrane structure, and increase the lipid peroxidation reaction and DNA damage (Malar et al. 2015a, b). The molecular and biochemical mechanism of Hg genotoxicity remains unexplained but the main reason behind is overproduction of ROS (Deng et al. 2013). Mercuric chloride was more toxic in comparison to mercurous chloride. Likewise in one of the previous reports the seeds of *Hordeum vulgare* were found to be less effected than *Allium cepa* when exposed to mercuric chloride for a short duration (Patra et al. 2004).

2.4 Cadmium

Cadmium (Cd) is a toxic metal that enters in the environment mainly by industrial processes and is then transferred to the food chain by plants (Lin et al. 2007). Cd accumulation in plants leads to generation of ROS (H_2O_2 and O^{2-}) resulting in damage of cellular components such as proteins, lipids, and DNA (Lopez et al. 2006). Cd inhibits DNA-repairing enzymes and reacts with nucleotides resulting in DNA damage (Jin et al. 2003). Cd also induces the production of ROS that leads to lipid peroxidation and causes membrane disruption (Lin et al. 2007). Cd induces DNA damage or modifications include SSBs, DSBs, base and sugar lesions, abasic sites, and DNA-protein cross-links (Roldán-Arjona and Ariza 2009).

There are several reports on Cd-induced genotoxicity in plants in previous literature such as in *Arabidopsis thaliana* (Liu et al. 2008), *Zea mays* seedlings (Mohsenzadeh et al. 2011), *Vicia faba* roots (Taspinar et al. 2009), and *Hordeum vulgare* roots (Liu et al. 2009). In *Arabidopsis thaliana*, Cd induces DNA mismatch repair at lower concentration but repressed at higher concentrations (Liu et al. 2008). The presence or absence of DNA fragments in Cd-treated plant samples compared to control may be due to mutations, which create new primer-binding sites for Cd in *Brassica juncea* (Fusco et al. 2005).

2.5 Chromium

Chromium (Cr) is the seventh most abundant metal contaminant found in atmosphere due to its huge industrial utilization (Panda and Choudhury 2005). In nature, Cr exists in two different forms, trivalent chromium (Cr^{III}) and hexavalent chromium (Cr^{VI}). Phytotoxicity caused by Cr has been investigated by several authors on different crop plants as well as some lower plants (Hayat et al. 2012; Ali et al. 2013). Cr^{VI} is much more toxic when compared to the Cr^{III} (Han et al. 2004).

Cr causes plant growth inhibition due to cell division inhibition by inducing chromosomal aberrations (Liu et al. 1993). Cr reacts with DNA either directly (Krepkiy et al. 2001) or indirectly by inducing ROS activity (Shi et al. 1994). There are three different proteins involved in DNA damage caused by Cr. One is putative ubiquitin-conjugating enzyme, second is thiamine thiazole synthase 2, and third one is thiamine thiazole synthase 1 in maize leaves (Wang et al. 2013). Zou and colleagues demonstrated the effects of Cr^{VI} on root cell division of *A. viridis* and concluded decreased mitotic index (Zou et al. 2006).

2.6 Copper

Copper (Cu) is an essential micronutrient, acts as a cofactor in several enzymes, and is essential in different physiological mechanisms such as cell wall metabolism, protein synthesis, and lipid and nucleic acid metabolism (Cuypers et al. 2002). At higher concentration, Cu causes ROS generation (O^2, OH, O^{1-}, H_2O_2) which leads to cellular redox imbalance (Gill and Tuteja 2010).

The extent of Cu damage is determined by tail movement of fragmented DNA. At higher concentration Cu showed DNA damage. ROS generation is the major cause of Cu-induced DNA damage in plants. It causes oxidative damage to nuclear, chloroplastic, and mitochondrial DNA. Cu-induced DNA damage is caused either by direct DNA strand breaks (SSBs and DSBs) or by binding with some important enzymes which are involved in DNA replication, transcription, and repair (Liu et al. 2007).

2.7 Cobalt

Cobalt (Co) stress in plants causes severe damages to plant cell by inducing disruption in cell membrane, and reducing growth and biomass concentration (Pandey et al. 2009). Plants can take up small amounts of Co from the soil, controlled by several mechanisms. Co absorption by plant roots involves the active transport across cell membranes, although its molecular structure is not yet well understood (Bakkaus et al. 2005). Only limited studies are available on the Co toxicity in higher plants. Co is toxic only in high concentration (Osman et al. 2004).

Co causes apoptotic DNA damage, but its complete mechanism is still unknown; they may directly react with DNA and cause viability changes in cell and compromise of DNA integrity. Sometimes Co indirectly reacts with DNA by generating ROS (Gopal et al. 2003). Previous findings showed the appearance of DNA fragments after Co exposure supporting the assumption that excess Co indirectly mediates DNA degradation.

3 Genotoxicity Action Mechanism

Complete action mechanism at the molecular level of genotoxicity in plants is unknown, even though the lethal effects of heavy metals on crop have long been recognized. Most of the heavy metals are lethal at particular concentrations, due to their high affinity for thiol groups. Clastogenic effect of heavy metals depends on the concentration and its exposure duration. In plant systems when these heavy metals interact with plasma, there is change in viscosity, leading to the formation of chelated complexes and resulting in spindle dysfunction. Complete mechanism of heavy metal causes genotoxicity in plants that is described in Fig. 1.

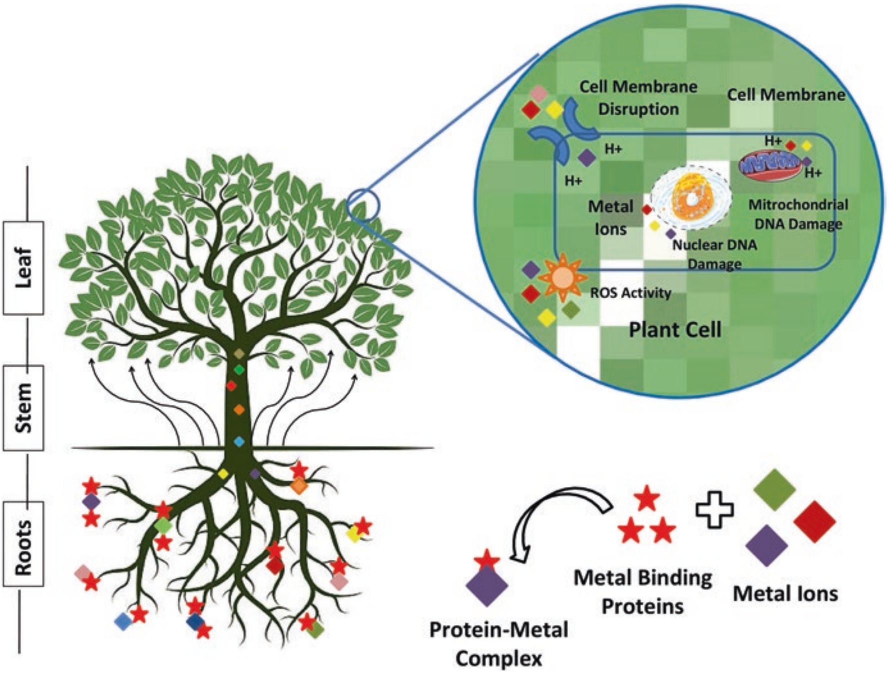

Fig. 1 Heavy metal-caused genotoxicity mechanism in plants

The first step of heavy metal interaction with plant cell is the mineralization of the membranes and cell walls. Next step is the microtubule disruption, which is essential for mitosis. Exposure of heavy metal effects in the G2 and M phase of cell division leads to the formation of abnormal cells (Hartwig et al. 2002). This phenomenon occurs due to the interaction of heavy metals directly or indirectly with the proteins involved in the cell cycle (e.g., cyclins).

Furthermore, low concentration of heavy metal induces chromosomal aberrations in plant cell (Pourrut et al. 2011). These chromosomal aberrations involve the formation of chromosome bridges, formation of micronuclei, microsatellite instability, loss of eccentric fragments, chromosome fragmentation, SSBs, and DSBs (Gichner et al. 2008; Azevedo and Rodriguez 2012).

Many of the genotoxic effects showed by heavy metal in plant cells are mediated by ROS activity (Ahmad et al. 2008). Some heavy metals directly bind to DNA or protein associated with them and cause DNA disruption (Pourrut et al. 2011) and some others have strong affinity to bind thiol groups of several proteins such as DNA polymerases, glutathione reductase, DNA ligases, and topoisomerase II (Aina et al. 2007).

4 Detection of DNA Damage

Cytogenetic tests analyze the frequency and type of DNA damage in plant cells. Heavy metals cause DNA damage, which leads to alterations in DNA. There are several tests available to detect DNA damage in plants; some are described below.

4.1 Allium Test

Allium test is one of the conventional methods to detect chromosomal aberration in plants. This method was developed by Levan in 1938 (Levan 1938). This method uses root tips from Allium bulbs, due to having eight pairs of large chromosomes, which allow the easy detection of any chromosomal aberrations. Micronuclei are the extranuclear component formed as a consequence of chromosome aberrations. The frequency of cells with micronuclei proves to be a good indicator of the genotoxic effect on plants (Leme and Marin-Morales 2009).

4.2 MNC Test

Micronuclei (MNC) test is also one of the conventional methods for the detection of DNA damage. It uses either mitotic cell of root tips (*Allium cepa* and *Vicia faba*) or meiotic tetrad cells of *Tradescantia*. *Tradescantia* is mainly useful for the detection of hazardous conditions in the environment (Mišík et al. 2011). MNC test is mainly of two types: the micronucleus assay (Trad-MCN) and stamen hair mutation (Trad-SH) test (Ma et al. 1994).

The micronucleus test, in *Tradescantia* (Trad-MCN) mother cells of pollen grains, estimates genetic damage, in chromosomal fragments or whole chromosomes that can be transmitted to next generations (Guimaraes et al. 2000). The micronucleus test is conducted in a study to determine the mutagenic effects of heavy metals in *Tradescantia*. Heavy metal treatment significantly increases the micronuclei frequency in mother cells of pollen grains. Trad-MCN is more sensitive than *A. cepa* bioassay for the detection of DNA damage in plants.

4.3 Molecular Markers

The conventional methods use different plants such as *Glycine max*, *Allium cepa*, *Zea mays*, *Hordeum vulgare*, *Vicia faba*, and *Arabidopsis thaliana* for the detection of genotoxic effect on plants. Nowadays several new methods are employed for the detection of DNA damage in plants like molecular markers, comet assay, chromosome aberration assay, and micronucleus assay (Collins 2000).

The development of molecular marker technology has provided new tools for the detection of DNA damage in plants directly at the level of DNA. A dominant marker named random amplified polymorphic DNA (RAPD) is efficient for DNA analysis, cost effective, and less time consuming. It yields information on a large number of loci without having the prior knowledge of gene sequence (De Wolf et al. 2004). The DNA damage can be analyzed by reading-obtained band pattern such as missing bands, band shifts, and appearance of new bands. Molecular markers are used to detect different types of DNA damage such as rearrangements, small insertions or deletions of DNA, point mutation, and ploidy changes (Atienzar et al. 2000).

4.4 SCE Test

The sister chromatid exchange (SCE) test is highly sensitive for the detection of DNA damage in plants due to heavy metal effect. The test is based on DNA segregation, which involves the symmetrical exchange of sister chromatids without alteration of genetic information and length of chromosome. Sister chromatids are visualized through staining methods (Painter 1980). The genotoxic effect of heavy metals in plants is showed by the increase in frequency of SCEs per chromosome. SCE test can be employed for both plant and animal cells. Plant species used for SCE test are *Allium cepa* and *Vicia faba* (Cortes et al. 1987).

4.5 *Comet Assay*

The comet assay is one of the important and well-known techniques for the detection of DNA damage in plants and animal cells. Comet assay is quantified by the concentration of migrated DNA, which migrates out of the nuclei toward the anode in a thin layer of agarose gel during electrophoresis. After DNA migration the nuclei appear to have a "comet-like" shape with staining (e.g., ethidium bromide). The comet assay was first proposed in 1984 by Östling and Johanson (Ostling and Johanson 1984).

Different modification in comet assay was described by Angelis et al. (Angelis et al. 1999) who employed combinations of neutral and alkali pH solutions. Treatment of DNA to high alkali before electrophoresis under neutral conditions allows the detection of SSB (Yendle et al. 1997). DSBs are detected via comet assay under completely neutral conditions. For the detection of abasic sites DNA is treated with endonucleases after lysis (Angelis et al. 2000). The comet assay has also been applied to onion (Navarrete et al. 1997), *Vicia faba* (Koppen and Verschaeve 1996), tobacco (Gichner and Plewa 1998), carrot (Zhen et al. 1998), *Impatiens balsamina* (Poli et al. 1999), and barley (Jovtchev et al. 2001).

4.6 *TdT-Mediated dUTP Nick End Labeling (TUNEL)*

Another test used for the detection of DNA damage or chromosomal aberration in plants is the TUNEL test (Martins et al. 2007). The advantages of the TUNEL test include detection of DNA breaks at a single nucleus, and being less time consuming and much user friendly. This test is recommended for the preliminary evaluation of genotoxicity of any new tested agent (Havel and Durzan 1996).

The fluorochrome-based TUNEL assay is applicable for flow cytometry, combining the detection of DNA strand breaks with respect to the cell cycle-phase position (Oliveira et al. 2011). Since 1992 the TUNEL has become one of the main methods for detecting DNA damage in plants with heavy metal side effects.

5 DNA Damage

5.1 *Oxidative Injury*

Heavy metal accumulation in plants caused severe damage to its DNA and protein contents (Jaishankar et al. 2014). Different kinds of DNA damage caused by heavy metals in plants are described in Fig. 2 and Table 1. Heavy metal directly interacts with photosynthetic machinery of the plant, resulting in photooxidative and genotoxic damage. Photosynthetic machinery of plant includes several concerned

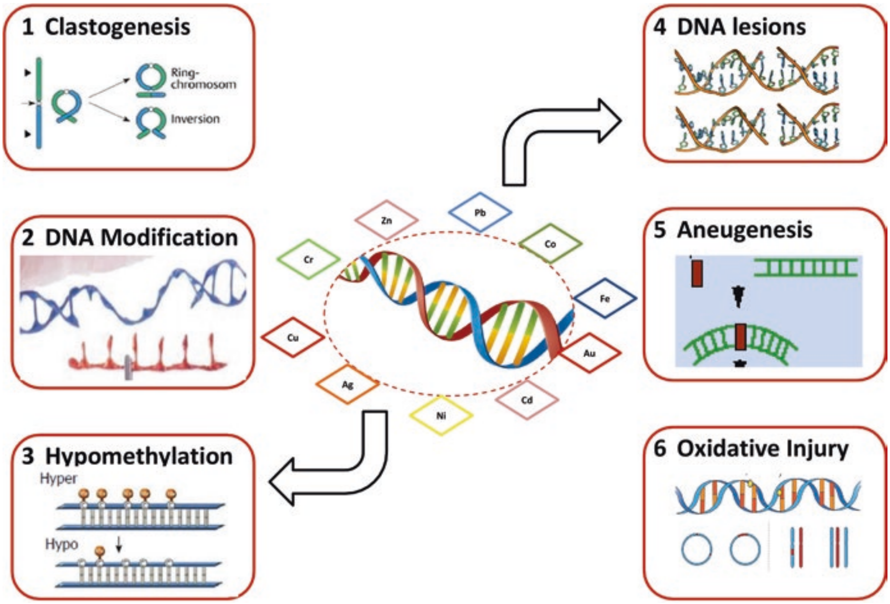

Fig. 2 Different kinds of DNA damage caused by heavy metals in plants

enzymes, proteins, genetic materials, chlorophyll, and carotenoid components (Zengin and Munzuroglu 2005). Oxidative damage to plant is related to the production of reactive oxygen species (ROS) which is important for the synthesis of several low-molecular-weight compounds such as carotenoids and other pigments (Ahmad et al. 2009).

ROS are the major source for the DNA damage in plants (Das and Roychoudhury 2014). ROS can damage all kinds of genetic material present in plants which include nuclear, chloroplast, and mitochondrial DNA (Sharma et al. 2012). Oxidative damage to genetic material of plants results in DNA lesions (SSBs and DSBs), removal of nucleotides, DNA modifications, DNA oxidation, DNA-protein cross-links, etc. (Bertin and Averbeck 2006). Oxidative damage to plant genetic material generally involves the following:

- Addition of hydroxyl group (OH) to the DNA double bonds which directly affects the deoxyribose backbone and purine/pyrimidine bases (Halliwell and Gutteridge 2015).
- Further addition of OH generates various products from the DNA bases such as urea, hydroxymethyl urea, thymine, thymine glycol, and some other saturated products. The reaction mechanism involved in this is hydroxylation of guanine (Tsuboi et al. 1998).
- Several mutagenic alterations of DNA include modification of G:C sites (Sharma et al. 2012).

Table 1 Different kinds of DNA damage caused by heavy metals in different plants

Heavy metals	Concentration in plants (µg g^{-1})	Plants	DNA damage	References
Lead (Pb)	1.0	*Vicia faba*	Clastogenic, aneugenic	Pourrut et al. (2011)
		Nicotiana tabacum	DNA-protein cross-links	Gichner et al. (2008)
		Talinum triangulare	Single-stranded and double-stranded DNA breaks	Kumar et al. (2013)
Chromium (Cr)	1.5	*Brassica napus*	Hypermethylation	Labra et al. (2004)
		Vicia faba	Chromosomal aberrations	Koppen and Verschaeve (1996)
Iron (Fe)	150	Random plants	Single-strand DNA break	Sestili et al. (2002)
		Pisum sativum	Oxidative injury	Floyd et al. (1989)
Gold (Au)	0.001	–	–	–
Copper (Cu)	10	*Pisum sativum*	Oxidative injury	Hattab et al. (2009)
		Random plants	DNA strand cleavage	Maksymiec (1998)
Cadmium (Cd)	0.05	*Vicia faba*	Single-strand DNA break	Lin et al. (2007)
		Nicotiana tabacum	Oxidative injury	Gichner et al. (2004)
		Solanum tuberosum	Necrotic DNA fragmentation	Gichner et al. (2008)
		Hordeum vulgare	DNA fragmentation	Liu et al. (2005)
Silver (Ag)	0.2	*Allium cepa*	Oxidative injury	Cvjetko et al. (2017)
Mercury (Hg)	0.1	*Eichhornia crassipes*	DNA modification	Malar et al. (2015a)
		Sesbania grandiflora	Oxidative injury	Malar et al. (2015b)
		Mentha arvensis	DNA strand break	Manikandan et al. (2015)
Manganese (Mn)	200	*Phaseolus vulgaris*	DNA modification	Enan (2006)
Cobalt (Co)	0.2	*Brassica juncea*	Apoptotic DNA damage	Karuppanapandian and Kim (2013)
		Zea mays	Inhibit DNA repair enzyme	Erturk et al. (2013)
		Allium cepa	Chromosomal aberrations	Yıldız et al. (2009)
Nickel (Ni)	1.5	Random plants	Oxidative injury	Chen et al. (2009)

(continued)

Table 1 (continued)

Heavy metals	Concentration in plants (µg g^{-1})	Plants	DNA damage	References
Zinc (Zn)	50	*Oryza sativa*	Nuclease activity	Cantos et al. (2014)
		Cassia angustifolia	Oxidative injury	Nanda and Agrawal (2016)
		Nicotiana tabacum	DNA-protein cross-links	Procházková et al. (2013)
Aluminum (Al)	80	*Allium cepa*	DNA strand break	Murali Achary and Panda (2009)
		Oryza sativa	DNA strand break	Meriga et al. (2004)
		Hordeum vulgare	Chromosomal aberrations	Pan et al. (2004)
		Allium cepa	Oxidative injury	Achary et al. (2012)
Molybdenum (Mo)	0.5	–	–	–
Antimony (Sb)	0.0005	*Vicia faba*	Clastogenic	Steinkellner et al. (1998)
Boron (B)		*Malus domestica*	Nuclease activity	Molassiotis et al. (2006)

- Single-strand breaks (SSBs) can be caused due to attack on DNA sugar molecules. ROS removes a hydrogen atom from deoxyribose, leading to generation of a deoxyribose radical that results in SSBs of DNA molecule (Evans et al. 2004).
- Formation of DNA-protein cross-links takes place when OH radicals directly attack either DNA or protein associated with them. It cannot be readily repaired and may be lethal (Reardon et al. 2006).

5.2 Hypomethylation

The plant genome approximately contains 30% of 5-methylcytosine of total cytosines in DNA molecule, but it can vary from species to species (Steward et al. 2002). Regulatory mechanism involves DNA methylation process. Formation of heterochromatin depends on the proper methylation of DNA and histone tails (Peters and Schübeler 2005). Several studies showed that alteration in DNA methylation resulted in serious modification of gene expression and its transcriptional products (Bender 2004). DNA methylation involves different enzymes such as DNA methyltransferase 1, chromomethylase, and domain-rearranged methyltransferase (Wada et al. 2004).

In higher plants the process of DNA methylation is more complex in comparison to animals (Vanyushin and Ashapkin 2011). It involves a larger set of specific DNA methyltransferases, some of which have no analogs in animals (Vanyushin and

Ashapkin 2009). The methylation and demethylation at GC sites involve an enzyme named DNA methyltransferase 1 (MET1). Some other enzymes are also related to DNA methylation such as deficient in DNA methylation 1 (DDM1) and methylcytosine-binding proteins (VIM1, VIM2, VIM3) (Vanyushin and Ashapkin 2011). DNA methylation plays an important role in several fundamental cellular activities including maintenance of genomic integrity, control of genomic imprinting, control of gene expression, and formation of chromatin structure (Moore et al. 2013).

5.3 Clastogenesis

Clastogenic DNA damage means chromosomal/chromatid break. By clastogenic effect the terminally deleted chromosomes get placed which results in the formation of bridges and sticky chromosomes (Rank and Nielsen 1997). Induction by heavy metals of chromosomal bridges in ana-telophase is a firm evidence of clastogenicity (Borboa and De la Torre 1996).

Clastogenic DNA damage is a prolonged continued reaction producing broken ends in the chromosomes and bridge formation in ana-telophases (Borboa and De la Torre 1996). Due to the kind of heavy metal stress some silent genes get activated by plant genomes in response. Clastogenic DNA damage produces chromosomal stickiness via damage to chromosomal peripheral proteins (DNA topoisomerase II), which may also lead to chromosome breakage aberrations (Boos and Stopper 2000).

5.4 DNA Lesions

DNA lesions are mainly of two types: single-stranded breaks (SSBs) and double-stranded breaks (DSBs). SSBs are comprised of lesions only in one DNA strand, such as base damage (oxidized or alkylated), base loss, intra-strand cross-links, DNA photoproducts, and DNA adducts. DSBs include lesions on both of the DNA strands such as inter-strand cross-links (Kozak et al. 2009).

The mechanism behind DNA lesions is the increase in the concentration of free radicals around the DNA (Sharma et al. 2012). DNA lesions occur at apurinic/apyrimidinic (AP) sites by spontaneous hydrolysis of the N-glycoside bond (Tuteja et al. 2009). Several factors are involved in inducing DNA lesions such as monofunctional alkylators MMS and EMS (Natarajan 2005), ionizing radiation in the form of gamma and X-rays (van Harten 1998), radiomimetic agents (Stolarek et al. 2015), restriction endonucleases (Bryant 1990), and ultraviolet radiation (Britt 2004).

5.5 Inhibited DNA Repair Enzymes

The causative agent for DNA damage is either endogenous or exogenous but different types of DNA repair systems also exist to keep the mutation frequencies low. ROS is considered as one of the major sources of endogenous damage to DNA which include deamination, nonenzymatic methylation, spontaneous hydrolysis of DNA bases, etc. (Lindahl 1993). Damaged DNA repair mechanism involves the releasing of damaged base and generating sites of base loss (Friedberg et al. 2005). Some important enzymes used in DNA damage repair mechanism and their inhibition effects are discussed below:

5.5.1 DNA Glycosylases

DNA glycosylases are of two types: monofunctional, which remove the modified base off by cleaving and leave an abasic lesion, and bifunctional, which remove the lesion and then cleave the DNA at the abasic site (Hegde et al. 2008). Some small molecules are found which cause inhibition of DNA glycosylases such as Trp-P-1 and 2-thioxanthine (Jackson and Loeb 2001). Both the inhibitory molecules act as potential substrates for DNA glycosylase and bind at its active site.

5.5.2 DNA Polymerase β

The second and the most important enzyme in DNA repair is DNA polymerase β; it acts upon the DNA backbone repair (3′-hydroxyl and 5′-deoxyribose-phosphate termini). The mechanism of DNA repair using DNA polymerase β involves both its lyase and polymerase activity. The lyase activity involves the trimming of 5′-deoxyribose terminus to a phosphate and with the polymerase activity it inserts the appropriate complementary base into the vacant position.

Several molecules are reported which inhibit both the lyase and polymerase activities of DNA polymerase β (Jaiswal et al. 2009). Some natural products act as inhibitory agents against DNA polymerase β such as edgeworin, oleanolic acid, myristinin, and harbinatic acid (Gao et al. 2008). The action mechanism involved in DNA polymerase β inhibition is binding in the same region of DNA polymerase β based upon molecular modeling studies.

5.5.3 Apurinic Endonuclease

As we have mentioned above, in terms of the action mechanism of DNA glycosylase enzyme in DNA repair, it produces an abasic site after cleaving mismatched base and these sites have no coding information. It is estimated that around 10,000 abasic sites are produced per day per cell without any damage repair (Nakamura and Swenberg 1999). Apurinic endonuclease enzyme has the ability to repair these abasic sites (Xanthoudakis and Curran 1996). The inhibition of apurinic endonuclease enzyme can cause apoptosis resulting in immediate cell death (Chang et al. 2011).

Several apurinic endonuclease inhibitors have been reported in previous studies (Bapat et al. 2010). Many of these compounds mimic the diphosphate linkage present in the DNA (Mohammed et al. 2011). Example of apurinic endonuclease inhibitor is lucanthone, which inhibits via intercalation (Luo and Kelley 2004).

5.5.4 DNA Ligase

The last enzyme involved in DNA damage repair is DNA ligase; it ligates 5′-phosphate and 3′-hydroxy groups at the nick (SSBs). DNA ligase-I and ligase-III act as a scaffold that facilitates the repair of DNA damage (Pascal et al. 2004).

Some small molecules are initially screened in silico to inhibit the function of DNA ligase (Pascal et al. 2004). The mechanism behind the inhibition of DNA ligase is the binding of these inhibitors on the surface of DNA-binding domain of the protein. Four different compounds (DNA ligase inhibitor 67, 82, 189, and 197) are found, which inhibit ligase activity, but their action mechanisms are different (Chen et al. 2008). DNA ligase inhibitor 197 is the most active compound; it mimics apurinic endonuclease-1; on the other hand compounds 67 and 189 act as competitive inhibitors and compound 82 works as a noncompetitive inhibitor (Sun et al. 2001).

6 Conclusion

In this chapter we discussed the effects of some heavy metals, i.e., lead, copper, arsenic, mercury, cadmium, aluminum, and chromium, on plant DNA. Most of the cellular and molecular aspects of heavy metal genotoxicity in plants are unknown, even though deleterious effects on food production have long been recognized. Taken together, these observations suggest that genotoxic effects could be in part responsible for metal phytotoxicity, deserving more work to elucidate the underlying mechanisms.

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Heavy Metal-Contaminated Soils: Weeds as Potential Phytoremediation Agents—Issues and Prospects

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Contents

1 Introduction

Toxic heavy metals and metalloids which have no apparent role in plant nutritional requirements are regarded as emerging threats to ecosystems. Lead (Pb), cadmium (Cd), mercury (Hg), arsenic (As), and chromium (Cr) are among the toxic elements which serve as soil pollutants and harmful agents to living organisms (Duruibe et al. 2007; Chen et al. 2018; Majeed et al. 2019). Based on their natural origin and extensive human activities, though the risk of toxicity associated with heavy metals is variable in different regions of the world, it is apparent that intensification of agricultural soils with these metals and metalloids results in contamination of food chain and ultimately imparts adverse effects on human health. Although it is

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difficult to estimate the exact global land area which is polluted with heavy metals, different studies however suggest that the problem of heavy metal contamination in soils is severe and it will become more intense in the future as the pressure for attaining more production from agriculture increases (Zarcinas et al. 2004; Chen et al. 2015; Tóth et al. 2016).

Recovery of heavy metal-contaminated soils widely employs popular approaches such as physical separation, restriction, reducing of toxicity, solidification, and isolation of heavy metals among many others; however, the success of each of the particularly employed method depends on the concentration and nature of heavy metals and polluted sites (Mulligan et al. 2001). Feasibility, economic costs, and environmental outcomes remain the leading factors in the implementation of soil decontamination techniques. To devise practical, economically acceptable, and environmentally sound HM decontamination techniques, quests for novel remediation approaches are extremely necessary because the development of eco-friendly and economically feasible methods may lead to reduced reliance on costly and ambiguous traditional approaches which are used to recover heavy metal-polluted soils.

Phytoremediation—a technique which uses plants and/or microbes to reduce the toxic effects of heavy metals and to reclaim polluted soil—is an eco-friendly, economically efficient, and comparatively easy to be employed method in most of the contaminated sites which has drawn favorable responses from the scientific community (Wuana and Okieimen 2011; Ali et al. 2013). Phytoremediation is generally carried out in polluted sites by plantation of plants and amendments with specific microorganisms which exhibit a high degree of tolerance to heavy metals and metal extraction capacities. The advantages of phytoremediation over traditional methods are numerous. It provides a cost-efficient approach towards cleaning of contaminated soil by extracting both organic and inorganic effluents besides its appropriateness in terms of practice and sustenance of the environment. In earlier experiments, the effectiveness of many plants in remediating metal-contaminated soils has been demonstrated (Khan et al. 2000; Marchiol et al. 2004; Gupta and Sinha 2006; Zhuang et al. 2007). In comparison to cultivated plants, the significance of weeds in phytoremediation is more promising as weeds are not directly consumed by humans and animals, thus reducing the chances of heavy metal flow into the food chain. Weeds also do not require additional costs of purchase while for cultivated plants, seeds are purchased from seed centers. In literature, there are plenty of reports describing the uses of weeds in phytoremediation of heavy metals (Lum et al. 2014; Hammami et al. 2016; Ayeoffe Fontem and Chikoye 2017; Chandra et al. 2018). The aim of this chapter is to further elaborate our understanding of the phytoremedial potentials of weed plants.

2 Phytoremediation: Applications and Mechanism

Phytoremediation is a general term which is widely used to describe the methods which encompass the use of living organisms for removing heavy metals from the polluted soils and improving soil's physical and chemical properties which are supposed to be devastated by heavy metal contamination. A diverse range of plant species, microorganisms, lichens, and fungi are employed as phytoremedial agents though the remediation potentials of different organisms vary to different extents (Bačkor and Loppi 2009; Tangahu et al. 2011; Sepehri et al. 2013; Ullah et al. 2015; Majeed et al. 2018). Phytoremediation techniques are quite popular and widely applied in the developed countries; however, they are not yet fully adopted in developing countries which needs emergent consideration.

There are several factors which determine the efficiency of living organisms in the phytoremediation process. First, the tolerance level of plants, fungi, and microbes plays a key role in their selection for decontamination of polluted soil. In nature, plant species show differential tolerance to different metals and their varying concentrations. Some plant species are more tolerant to heavy metal stress because of their adaptive mechanisms which they have evolved and hence they are more ideal candidates for phytoremediation process. Secondly, the nature of pollutants, e.g., type of heavy metals and their concentration, strongly influences growth, physiology, and survival of plants which determine the suitability of some plants as more ideal for phytoremediation than others. Zn, Cu, Hg, Cr, Co, Cd, and Pb have been reported in a number of studies to exhibit extremely toxic effects on plant growth and developmental processes when their concentration increased beyond threshold levels (Yadav 2010). Thirdly, the ecology of polluted sites also regulates which plant species to be used in those areas for decontamination of heavy metals. Ideally, mesophytic plants with phytoremediation potential can work better in polluted sites which have the same characteristics. Similarly, aquatic, wetland, and xerophytic species are suitable for decontamination in their respective habitats. Fourthly, the use of plants and other living organisms either alone or in combination with other remediation methods also holds a key position in making the decontamination process a success.

A simple mechanism of the phytoremediation process is depicted in Fig. 1. Plants, microbes, fungi, lichens, and mycorrhizae which can withstand extra-threshold levels of heavy metal pollutants contribute to soil improvement by taking up heavy metals, accumulating them in shoots and plant parts, converting them to less toxic compounds, and releasing them as volatiles (Jabeen et al. 2009). Important phases in phytoremediation are the extraction and phytoaccumulation of metals from the soil by selected plants (Muthusaravanan et al. 2018). Roots absorb heavy metals and translocate them to shoots and aboveground parts. Phytoextraction may be continuous (accumulation and removal of pollutants by plants continuously) or induced (removal of toxins in a single time by the additions of chelators) (Peer et al. 2005). Extraction of heavy metals by plants leads to reduced metal load in contaminated soil and subsequently the soil becomes suitable for the growth of plants, microbes, and other living organisms. Plants which can tolerate and accumulate high metal load are termed as hyperaccumulators (Rascio and Navari-Izzo 2011). Toxic effects of heavy metals in shoots and other parts are reduced by either metabolic processes or activation of specific enzymes which involves several steps in a phase termed as phytodegradation (Peer et al. 2005; Muthusaravanan et al. 2018). In phytodegradation, cellular and enzymatic activities efficiently break down toxic compounds into simpler nontoxic ones (Suresh and Ravishankar 2004).

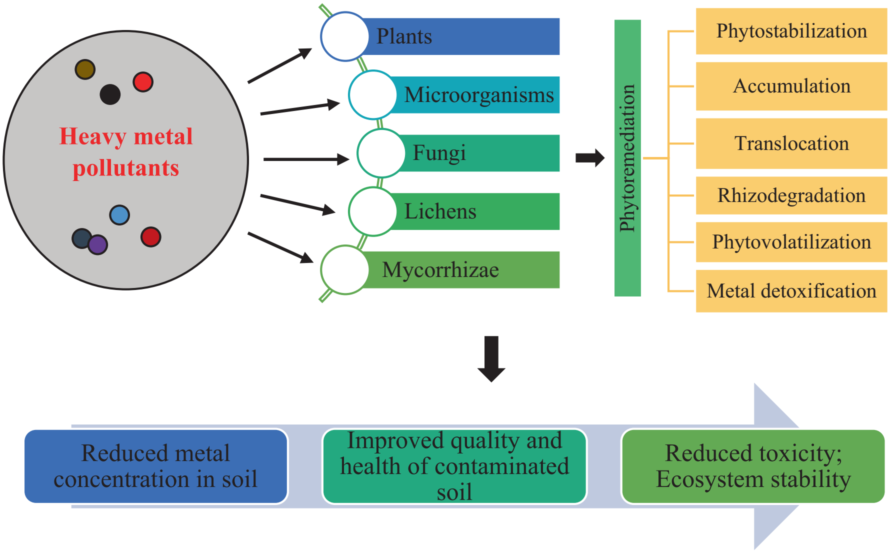

Fig. 1 A simple illustration of heavy metal bioremediation by plants, microbes, fungi, and lichens

Another step involved in phytoremediation is volatilization. Certain plants, specifically trees, have the ability to volatilize the extracted pollutants into the atmosphere (Pulford and Watson 2003). The phytovolatilization potentials of plants greatly vary among plant species while the nature of heavy metals and the acting metabolic processes inside harvestable tissues of plants also affect the processes of phytovolatilization. Although phytovolatilization of heavy metals generally occurs directly through leaves and stems of the plants, indirect volatilization from polluted soil may also proceed as a result of the rhizospheric and metabolic activities of the phytoextractants (Limmer and Burken 2016). Phytovolatilization is effective for the removal of volatile organic contaminants although other pollutants soluble in water may also be removed through this process. Arya et al. (2017) asserted that phytoextraction and volatilization occur in a simultaneous manner which can efficiently remove heavy metal pollutants such as mercury and other heavy metals.

Phytostabilization refers to the check on the mobility of heavy metals and pollutants in contaminated soil and stabilization of polluted soil through mechanical, chemical, or microbial support facilitated by suitable plants (Dary et al. 2010). Contaminated soil, if left unchecked, may further extend the pollution to non-polluted soils by water or air erosions which involve the mobility of heavy metals and other pollutants. Thus the stability of contaminated land and proper check on the mobility of heavy metals are necessary. It is achieved by maximum plantation in vulnerable soils. Plants grown in such a contaminated site serve three purposes: (1) they protect soil erosion through their roots, and canopy; (2) they leach out exudates and allelochemicals, and provide litter and mulch which can enhance soil's health; and (3) they stimulate microbial organisms to occupy the contaminated sites which are also active players in phytostabilization. Ideal plants for phytostabilization of polluted soils must have a deep and branched rooting system, tolerant to heavy metal stress, drought, waterlogging, and salinity and should possess great canopy (Mendez and Maier 2007).

3 Weeds and Their Potentials in the Phytoremediation Process

Weeds are plants that appear “undesirably” in soils and which compete with other plants for resources. They have been considered as a threat to the conservation of biodiversity, ecosystem services, and crop productivity (Ansong and Pickering 2015; Yaduraju et al. 2015). Although negative properties of weeds are apparent, they still have some useful aspects which can be utilized in a natural ecosystem and managed agriculture for addressing some ecological and agricultural issues. Like other plants, some of the weed species can serve in remediating heavy metal-polluted soils. Unlike other cultivated plants, weeds can be a good choice for phytoremediation because they exhibit several advantages over their cultivated counterparts which include their successive abilities and tolerance to adverse environmental stresses (Sharma and Pant 2018). Exploiting their tolerance to biotic and abiotic stresses, weeds are ideal candidates in the reclamation of soils polluted with heavy metals. In several studies, different weeds have been reported to exhibit phytoremediation potentials against a variety of metal contaminants (Table 1).

While selecting weeds for soil reclamation purposes, basic criteria such as their ability to tolerate heavy metal stress, accumulation of heavy metal in harvestable parts, stabilization and immobilization of heavy metals in soils, volatilization, and rhizodegradation are important components to be considered (Fig. 2). Ideal weeds should possess the extensive rooting system, broad leaves, and high biomass (Eapen and D’souza 2005). They should have excellent interaction characteristics with microbial communities since many of the microbes have confirmed properties in the improvement of soil’s health. Population dynamics of the rhizospheric microbes are determined by several factors among which the role of root exudates is a promising one. Roots can either attract microbes by the release of exudates, allelochemicals, and symbiosis or repel them by the negative allelopathy or antagonism. This would subsequently lead to either soil stability or further deterioration. In the context of heavy metal-polluted soils, positive interaction between potentially applied weed species and microbial communities becomes even more important because, without the contribution of microbes, phytostabilization will become less efficient. Thus, those weed species which can attract microbes and engage them in phytostability activities are more ideal agents in phytoremediation.

Tolerance to heavy metal stress is another important factor in the selection of weeds as phytoremedial agents. Not all the weed species are tolerant to heavy metals. Weeds like *Cardamine hirsuta* and *Gnaphalium affine* have been reported to

Table 1 A list of weeds with phytoremediation capabilities

Weed plants	Heavy metals	Phytoremediation properties	References
Pluchea indica	Cr	Phytoaccumulation	Sampanpanish et al. (2006)
Cyperus rotundus; Eicchornia crassipes	Cr	Phytoaccumulation	Sundaramoorthy et al. (2010)
Amaranthus spinosus	Cu, Zn, Cr, Pb, and Cd	Accumulation and translocation	Chinmayee et al. (2012)
Calotropis procera, Croton bonplandianum, Cyperus rotundus, and others	Cr, Cu, Ni, Pb, and Cd	Accumulation; soil restoration	Kumar et al. (2013)
Paspalum orbiculare, Eleusine indica, Dissotis rotundifolia, and *Kyllinga erecta*	Fe, Pb, Cr, Cu, Pb	Phytoextraction; phytostabilization	Lum et al. (2014)
Abutilon indicum	Cd	Phytoaccumulation; translocation	Varun et al. (2015)
Portulaca oleracea	Cr	Accumulation	Kale et al. (2015)
Taraxacum officinale; Solanum nigrum	Cd	Accumulation	Hammami et al. (2016)
Piper marginatum, *Sticherus bifidus*, and *Capsicum annuum*	Hg	Accumulation	Marrugo-Negrete et al. (2016)
Pennisetum americanum × *Pennisetum*, and *Sorghum dochna*	Cd, Pb, and Zn	Phytostabilization	Zhang et al. (2016)
Coronopus didymus	Pb	Accumulation	Sidhu et al. (2018)
Phragmites australis and *Arundo donax*	Different metals and pollutants	Phytostabilization	Castaldi et al. (2018)
Pistia stratiotes and *Eichhornia crassipes*	Cr, Cu	Accumulation	Tabinda et al. (2018)
Pterocypsela laciniata	Cd	Accumulation	Zhong et al. (2019)
Parthenium, Cannabis	Cr	Bioaccumulation	Ullah et al. (2019)
Solanum viarum	Cu, Pb, Zn, Cr, and Cd	Accumulation	Afonso et al. (2019)

tolerate Cd stress up to 100 mg kg^{-1} indicating their hyperaccumulating properties (Lin et al. 2014). In a comprehensive review, Ur Rehman et al. (2017) identified metal tolerance and accumulation properties of nightshade (*Solanum nigrum*) to different heavy metals. Other weed species in diverse plant families such as *Phragmites australis, Arundo donax, Pistia stratiotes, Eichhornia crassipes, Pterocypsela laciniata, Parthenium* sp., *Cannabis* sp., and *Solanum viarum* have been reported to exhibit metal tolerance and they may efficiently carry out phytoremediation in polluted soils (Castaldi et al. 2018; Tabinda et al. 2018; Zhong et al. 2019; Ullah et al. 2019; Afonso et al. 2019).

One of the several issues concerned with weeds' selection for bioremediation is the production of their low biomass, and narrow leaves. Ideal candidates should

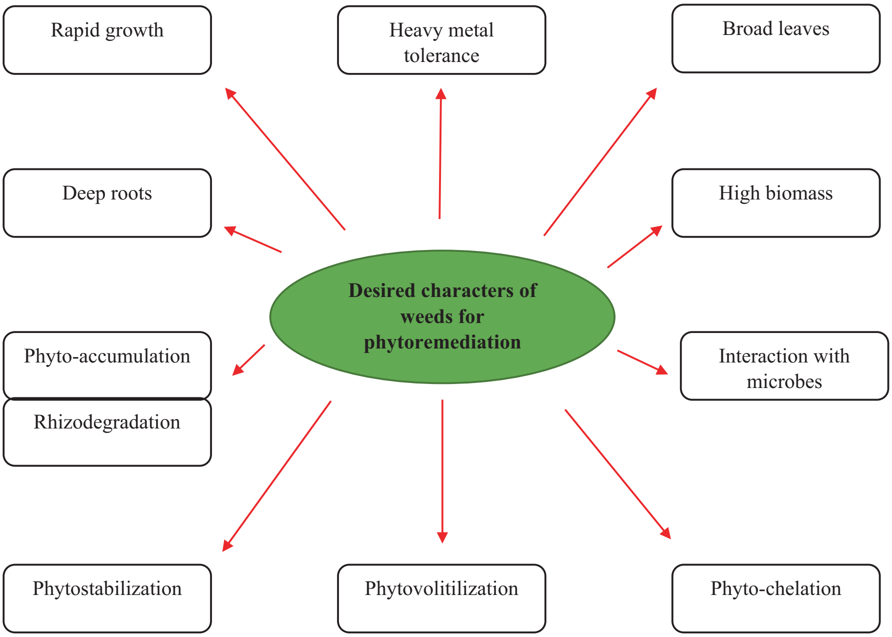

Fig. 2 Desirable characters of weed species for their selection in phytoremediation processes

possess broad leaves and high biomass as these characteristics will allow the weed species to accumulate greater quantities of heavy metals in their tissues. Cotton burdock (*Arctium tomentosum*) in the family Asteraceae is a broadleaf weed which has been suggested for phytoremediation of polluted soils because the weed can tolerate different heavy metals and can accumulate them in tissues to a significant extent (Harbawee et al. 2017). Other species with broad leaves and relatively high biomass include *Trifolium repens, Taraxicum officinalis, Vetiveria zizanioides, Typha latifolia,* and *Plantago major* and many others have been well studied for their phytoremedial properties (McDonald 2006; Danh et al. 2009; Wang and Oyaizu 2009; Bini et al. 2012; Romeh et al. 2016; Harbawee et al. 2017). A list of broadleaf weed species with potential application in phytoremediation is presented in Table 2.

4 Phytoremediation by Weeds: Challenges and Prospective Strategies

Although weeds have promising roles in phytoremediation of heavy metal-polluted soils, still some challenging issues restrict their use for the said purpose and they have not been fully exploited in phytoremediation techniques. A major problem associated with polluted sites is their suitability of growth for weeds. Some weeds

Table 2 A list of potent phytoremedial weeds which possess relatively broader leaves and high biomass than grasses[a]

Weed species	Family	Characters
Fallopia convolvulus	Polygonaceae	Annual herb, fast growth, leaves reasonably broad
Portulaca oleracea	Portulacaceae	Annual succulent
Chenopodium album	Chenopodiaceae	Annual weed
Taraxacum officinale	Asteraceae	Perennial herb
Plantago major	Plantaginaceae	Annual herb
Arctium minus	Asteraceae	Biennial shrub
Stellaria media	Caryophyllaceae	Annual herb
Rumex crispus	Polygonaceae	Perennial herb
Artemisia vulgaris	Asteraceae	Perennial herb
Amaranthus retroflexus	Amaranthaceae	Annual herb
Plantago lanceolata	Plantaginaceae	Annual herb
Capsella bursa-pastoris	Brassicaceae	Annual herb
Rumex acetosella	Polygonaceae	Perennial herb
Viola sororia	Violaceae	Perennial herb
Cirsium arvense	Asteraceae	Perennial shrub
Verbena bracteata	Verbenaceae	Annual herb

[a] https://plantscience.psu.edu/research/centers/turf/extension/plant-id/broadleaf

may find the polluted site as suitable for their growth while for others growth would not be possible. In soils with xeric properties, only xerophytic weeds would be required which delimit the efficiency of phytoremediation because all xerophytic weeds do not tolerate heavy metal stress nor they possess ideal characteristics such as broad leaves and high biomass. Similar issues occur with mesophytes and hydrophytes to be employed in respective heavy metal-polluted sites. Likewise, low biomass, poor root system, and slow growth rate of candidate plants are also limiting factors in the adaptability of phytoremediation as a widespread approach (Saxena et al. 2019). These problems are general with plants to be used in phytoremediation; weeds with similar properties, in particular, reduce the efficiency of phytoremediation techniques.

Phytoaccumulator plants have additional limitations of being specific to specific metals; thus a particular weed will not be appropriate for contaminated sites which possess multiple heavy metals (McIntyre 2003). Ali et al. (2013) highlighted “long time for cleaning, limited bioavailability of heavy metals, the concentration of heavy metals in contaminated soil, and the risk of food chain contamination” as some of the hurdles in phytoremediation. In weed-phytoremediation system, slowly growing plants can be replaced with fast-growing plants which significantly reduce the time required for cleanup. Screening of weeds for the accumulation of multiple heavy metals can make the process of phytoremediation a success. Moreover, identification of weed species which can tolerate excessive quantities of heavy metals offers a good choice for employment in phytoremediation. As for food chain contamination risks, weeds have advantages over other plants because they are not directly consumed by animals and humans.

5 Conclusions

Heavy metal contamination of soils presents environmental and ecological threats. Soils polluted with heavy metals are not suitable for growth and cultivation of crops. Consumable crops grown on polluted soils can result in food chain contamination and severe health abnormalities in man and animals. Most of the decontamination techniques employed in polluted soils are costly and not eco-friendly. Phytoremediation, which employs plants and microbes for reclamation of heavy metal-polluted soils, is an emerging technique which reduces costs and environmental degradation. The process encompasses different steps, i.e., phytoaccumulation, phytodegradation, phytostabilization, and phytovolatilization. Since cultivated plants are used by animals and human beings, their utilization in phytoremediation enhances the chances of food chain contamination. Weeds, on the other hand, do not pose such threats because they are undesirable plants and in most instances they are not used for domestic purposes. Unlike other cultivated plants, weeds possess high tolerance capacity to heavy metals and other environmental stresses. These properties make them ideal candidates for phytoremediation techniques. Selection of weed species as phytoremediation tools is an important factor which determines the success of phytoremediation. Ideal candidate weeds should be tolerant to heavy metals, fast growing, and possess high biomass and extensive roots. Employment of weeds in polluted soils for phytoremediation of heavy metals can reduce the risk of environmental pollution, reclamation of soils, and costs and further research in this area can lead to minimum reliance on traditional remediation approaches.

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Phytomanagement of Metal(loid) Polluted Soil Using Barley and Wheat Plants

M. Gil-Díaz and M. C. Lobo

Contents

1 Introduction

Environmental pollution is defined as the contamination of the physical and biological components of the earth and/or atmosphere system to such an extent that normal environmental processes are adversely affected (Muralikrishna and Manickam 2017). Pollution is a great and growing global problem due to its harmful effects on the ecosystem and human health. In fact, pollution is the largest environmental cause of disease and premature death in the world today and, in the most affected countries, health problems due to pollution are responsible for more than one death in four (Landrigan et al. 2018). The pollution of an ecosystem induces a decrease in living organisms living in it as well as in its biodiversity and productivity.

Pollution can be due to natural sources or anthropogenic activities which have increased since the nineteenth century due to industrialization and include mining and military activities, industrial processes, transports, electricity generation, urbanization, manufacturing, use of agrochemicals, and land application of domestic sludge (van Liedekerke et al. 2014). There is a lack of reliable data in registering polluted sites, especially in low- and middle-income countries, where the industrialization has increased the pollution levels. In the EU, it is estimated that there are 2.5 million potentially contaminated sites, of which about 14% (340,000 sites) require urgent remediation (van Liedekerke et al. 2014). On the other hand, the United States Environmental Protection Agency (USEPA) calculates that tens of

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thousands of sites in the country require remediation, and 1300 sites require urgent remediation (USEPA 2018). According to the Chinese Environmental Protection Ministry, 16% of all Chinese soils and 19% of its agricultural soils are polluted (CCICED 2015). In Australia, the total number of contaminated sites is estimated at 80,000 (DECA 2010). Factors such as population growth, technological advancement, and urbanization suppose a greater demand of natural resources. Any use of natural resources at a rate higher than nature's capacity to restore itself can result in pollution of air, water, and soil (Muralikrishna and Manickam 2017). USEPA estimated that it will cost up to $170–250 billion to clean up that nation's hazardous waste sites (USEPA 2004). According to the European Environment Agency, the management of contaminated land in Europe costs an estimated €6.5 billion per year.

2 Soil Pollution

Soil is a living system, open, self-organized in space and time, in constant evolution due to its interactions with rock materials, climate, and organisms, as well as with anthropogenic activities. The interaction between atmosphere, lithosphere, hydrosphere, and biosphere takes place in the soil. Thus, soil is vital to life on earth. It has an enormous functional and structural complexity due to the great diversity of both abiotic and biotic components as well as the processes that take place in it. It is worth noting that the biodiversity of soil is immense compared to aboveground biodiversity; for example, ten grams of soil contains about 10^{10} bacterial cells of more than 10^6 species. It has been estimated that the biodiversity of soil could make up as much as 25% of the total amount of described living species worldwide, although most of this diversity remains unknown (FAO and ITPS 2015). The contribution of soil biota to human life is of great importance because of stocks of soil biodiversity also representing an important biological and genetic resource for biotechnological applications (FAO and ITPS 2015).

The main components of the soil are mineral particles, organic matter, biological systems, water, and gases. The Soil Science Society of America formally defined it as the unconsolidated mineral or organic matter on the surface of the earth that has been subjected to and shows effects of genetic and environmental factors of climate (including water and temperature effects) and macro- and microorganisms, conditioned by relief, acting on parent material over a period of time. In addition, soil plays an important role in the water cycle and provides important ecological services.

Historically, soil science has been linked to its agronomic productivity, the capacity of the soil to produce crops. Soil degradation supposes a partial or total loss of soil productivity and functions. The soil has a certain buffering capacity against degrading phenomena. However, if the degrading processes exceed the buffer threshold, the soil will become degraded. Erosion, acidification, compaction, sealing, organic carbon loss, nutrient imbalance, waterlogging, salinization, loss of biodiversity, and contamination are the main causes of soil degradation. Soil can be considered as a nonrenewable resource due to the slowness of soil formation processes, whereas soil degradation often takes place much faster.

Soil pollution is one of the ten major soil threats identified in the 2015 Status of the World's Soil Resources (FAO and ITPS 2015). Chemical pollution implies the presence of certain chemical elements or potentially harmful substances in amounts that exceed recommended levels for the health of humans, animals, and plants or cause a detriment on soil normal functioning (Orgiazzi et al. 2016; Pierzynski et al. 2005). In this sense, one of the priority objectives of the EU is to protect, conserve, and enhance the Union's natural capital, and for that the EU is committed to sustainable land management, adequate soil protection, and remediation of contaminated sites (DECISION No. 1386/2013/EU).

Soil pollution can be local or diffuse; local soil pollution occurs where intensive industrial activities, inadequate waste disposal, mining, military activities, or accidents introduce excessive amounts of pollutants; whereas diffuse soil pollution can be caused by a variety of activities that have no specific point of discharge, it occurs where emission, transformation, and dilution of contaminants in other media have occurred prior to their transfer to soil (Jones et al. 2012). Agriculture is a key source of diffuse pollution, but urban land, forestry, atmospheric deposition, and rural dwellings can also be important sources. The pollutants can be organic or inorganic. The organic pollutants include mineral oils, chlorinated hydrocarbons (dioxins, furans, polychlorinated biphenyls, and other chlorinated solvents), polycyclic aromatic hydrocarbons, phenols, aromatic hydrocarbons, pesticides, and so-called emerging pollutants such as pharmaceutical and veterinary products, illicit drugs, personal care products, food additives, and engineered nanomaterials. The inorganic pollutants include metals such as Ag, Cd, Co, Cr, Cu, Hg, Ni, Pb, and Zn; metalloids (As, Sb, Se); radionuclides, and some ions as phosphate, ammonia, nitrate, sulfate, and cyanide. The most frequent pollutants in soil and groundwater are mineral oils and metals (van Liedekerke et al. 2014).

3 Soil Pollution by Metals and Metalloids

Metals and metalloids, also called metal(loid)s, are introduced in the environment due to natural and human activities including mining, industrial and military activities, transport, urban development, use of agrochemical, and land application of industrial or domestic sludge (Adriano 2001; Kabata-Pendias 2011). Unlike organic contaminants, metal(loid)s in the environment cannot be chemically degraded, and consequently persist in soil for extended periods after their introduction. In this sense, the persistence of these pollutants in soil is much longer than in other compartments of the biosphere, being able to be considered almost permanent (Kabata-Pendias 2011). The concentration of metal(loid)s accumulated in soils can decrease slowly by leaching, plant uptake, or soil erosion. Plants can absorb the metal(loid)s accumulated in the soil and transfer them into the food chain which pose a serious hazard for animal and human health. Accumulation of metal(loid)s can cause severe health problems including lung, kidney, liver damage, and cancer (Adriano 2001; Kabata-Pendias 2011). Arsenic affects the respiratory system, the circulatory system, the skin, the nervous system, and the reproductive system, among others (Zhang and Selim 2008); exposure to Pb induces adverse effects on cognitive development in children (Appleton et al. 2012); Cd causes serious illnesses such as itai-itai, affecting also kidney and respiratory system (Bolan et al. 2013; Godt et al. 2006); the uptake of methylmercury causes severe neurological damages, as the case of Minamata in Japan in 1959 (Kabata-Pendias 2011); Cr(VI) is much more toxic than Cr(III) and can affect liver, kidney, respiratory tract, gastrointestinal and immune systems, blood, and skin and is considered a human carcinogen (Unceta et al. 2010).

The capacity of plants for absorption of metal(loid)s depends on their characteristics and availability, plant species, soil properties, and environmental factors. The total concentration of metal(loid)s in soil provides little information on their potential impact on soil organism; the bioavailability, referred to their accessible fraction which can be assimilated by an organism, is a better indicator of the risk for biota (Kumpiene et al. 2017). In this sense, there is a certain tendency in using the concept of bioavailability in risk assessment and management of contaminated sites and some countries have introduced this concept in legislation (Kumpiene et al. 2017). There are a wide variety of analytical methodologies to determine the available fraction of metal(loid)s in soils, such as water, diluted acids (HCl), leaching methods (TCLP test, 1311 USEPA), neutral salt solutions ($NaNO_3$, $MgCl_2$, $CaCl_2$, NH_4NO_3, $Sr(NO_3)_2$), strong complexing agents (diethylenetriaminepentaacetic (DTPA), ethylenediaminetetraacetic acid (EDTA)), or a mixture of low-molecular-weight organic acids (lactic, acetic, formic, citric, and malic acids), which simulates the composition of soil solution in the rhizosphere. Due to their different chemical characteristics, metal(loid) availability depends on the used extracting agent. However, there is no consensus on recommended methodologies to evaluate the metal(loid) availability (Kumpiene et al. 2017). The bioavailability of metal(loid)s depends on the degree of pollution, metal(loid) characteristics, and soil properties including pH, electrical conductivity, cation exchange capacity, nature of organic and inorganic ligand ions, texture, and redox condition, and may vary depending on the environmental conditions. Therefore, in acidic conditions, the availability of Zn, Pb, Ni, Cd, and Cu could increase, whereas for As and Cr it could decrease (Kabata-Pendias 2011). Metal(loid)s can be found in soils under different forms, including (in decreasing order of availability) solubilized in the soil solution; exchangeable ions in the exchange complex; adsorbed onto oxides and hydroxides of aluminum, iron, and manganese; complexed with organic matter; and mineral soil fraction.

Toth et al. (2016b) evaluated the presence of metal(loid)s in soils of the EU and concluded that most of the examined elements remained under the corresponding threshold values in the majority of land of the EU. However, one or more of the elements exceed the applied threshold concentration on 1.2 M km^2, which is 28.3% of the total surface area of the EU. While natural backgrounds might be the reason for high concentrations on large proportion of the affected soils, historical and recent industrial and mining areas show elevated concentrations too (predominantly of As, Cd, Pb, and Hg). In addition, Toth et al. (2016a, b) in a study on metal(loid)s in agricultural soils from EU found that the majority of European agricultural land can be considered adequately safe for food production, whereas an estimated 6.24% or 137,000 km^2 needs local assessment and eventual remediation action.

4 Remediation of Metal(loid)-Polluted Soils

Nowadays, there are a wide variety of strategies of soil remediation based on physicochemical or biological technologies for metal(loid)-polluted soils. The physicochemical technologies can be in situ or ex situ and may have a great impact on the edaphic environment. Soil washing, solidification, electrokinetic methods, and immobilization strategies are the most studied physicochemical technologies to remediate metal(loid)-polluted soils. Soil washing is an ex situ technique based on an aqueous solution with different extractants (organic acids, chelating agents, surfactants, humic substances, and cyclodextrins) which dissolves or suspends the pollutants (Khalid et al. 2017). It can be used for organic pollutants and metal(loid)s. The extractant solution and polluted soil are mixed thoroughly for a specified time, so that the metal(loid)s in the soil are transferred to the aqueous phase through precipitation, ion exchange, chelation, or adsorption (Khalid et al. 2017). The decontaminated soil could be backfilled to the original site.

Vitrification is a solidification method in which an electric current is used to melt the soil at temperatures up to 2000 °C to form a homogeneous liquid phase and then it is cooled to generate a single-phase glassy product; virgin materials such as silica and calcium carbonate or glass scraps may be used as process additives (Astrup et al. 2016). It can be performed in situ or ex situ. This procedure destroys the soil, thereby limiting its future use. In addition, vitrification needs a high energy consumption, resulting to be more expensive than other physicochemical strategies or simple disposal in landfills. Thus, adoption of this method is likely to be limited to contaminants under extreme cases where pollutant risks are very high, such as for radionuclide-polluted soils (Lombi and Hamon 2005). The addition of immobilizing agents to soil to stabilize the available fraction of metal(loid)s can be considered a solidification/stabilization method (Kumpiene et al. 2008). These agents induce the immobilization of metal(loid)s by absorption, precipitation, or complexation; the mobility and bioavailability of metal(loid)s are reduced, decreasing their potential leaching to deeper soil layer and groundwater as well as soil phytotoxicity. Different natural or synthetic materials, such as phosphate compounds, carbonates, alkaline agents, zeolites, clay minerals, and different wastes (sewage sludge, by-products from the iron and aluminum industry, gypsum, and lime-rich industrial by-products), can be used to immobilize metal(loid)s in soils (Gil-Díaz et al. 2017; Gil-Díaz and Lobo 2018; Kumpiene et al. 2008; Querol et al. 2006). Recently, nanoscale zerovalent iron has been effectively used to reduce metal(loid) mobility in polluted soils (Bardos et al. 2018; Gil-Díaz et al. 2017, 2019; Gil-Díaz and Lobo 2018). Electrokinetic remediation is an in situ washing process in which an electrical field is created in a soil matrix by applying a low-voltage direct current to electrodes inserted into the ground. As a result of this electric field, metal(loid)s are mobilized using an extractant solution as citric acid; then they are concentrated at the electrodes, and extracted from the soil. The main advantages of electrokinetic remediation are its low cost compared with other physicochemical strategies, the applicability to a wide range of contaminants, and its speed (Lobo et al. 2014). Comparative costs of the remediation techniques are summarized in Table 1. There is considerable controversy regarding the costs of remediation strategies and they depend on the method used for calculation and the factors considered in relation to the pollutants and the site characteristics. In this sense, the Federal Remediation Technology Roundtable (FRTR) estimates a high cost to apply phytoremediation technologies at sites of varying size and complexity because in addition to staff costs and design technology, it includes a complete monitoring for 5–20 years.

Bioremediation is a biological technique which is based on the reduction of pollutant concentration by the action of living organisms including microorganisms and plants.

The use of microorganisms for soil remediation is mainly referred for the removal of organic pollutants. In this case, microorganisms are able to biodegrade organic compounds producing carbon dioxide and water releasing carbon and nitrogen to use them as a source of energy (Wilson and Jones 1993). Considering the adequate degradation route in order to avoid the formation of hazardous intermediate metabolites, soil could be clean after the use of a specific degrading microorganism (Niaz et al. 1996). The processes of bioremediation should consider both the removal of the contaminant and the maintenance/improvement of soil fertility (Haferburg and Kothe 2007).

However, when the pollutants are metal(loid)s, biodegradation is not possible, although microorganism capacities can be used as an interesting tool for soil remediation through processes of coagulation, bioprecipitation, biosorption, extracellular sequestration, chelation, and transport mechanisms (Unz and Shuttleworth 1996). Moreover, microbial metabolism and growth can lead to changes in metal(loid) solubility (Haferburg and Kothe 2007). In this sense, the use of some

Table 1 Estimated cost of different remediation technologies for metal(loid)-polluted soil

Technology	Estimated cost (US$/m^3)	References
Phytoremediation	38	Wan et al. (2016)
	147–2322	FRTR[a]
Electrokinetic	90–400	FRTR
Soil washing	70–187	FRTR
Vitrification	50–330	FRTR
Excavation and landfill	300–510	FRTR

FRTR Federal Remediation Technology Roundtable, a Remediation Technologies Screening Matrix and Reference Guide. Available: https://frtr.gov/matrix2/section4/4-3.html (Accessed 2 Sept 2019)

[a] In addition of staff cost, design technologies, and analysis of soil and plant samples, the method includes a complete monitoring between 5 and 20 years

microbial consortia has shown promising results in removing Cr(VI) from anthropogenically polluted soils (Aparicio et al. 2019). In recent years, advances in genetic engineering have allowed for the use of transgenic organisms in bioremediation assays. The genes involved in the degradation of a specific contaminant are manipulated to increase the production of specific enzymes. A modification can also be made to the sequence, for example to increase the degradability of a compound or the resistance to high concentrations of the metal(loid). However, currently the use of genetically modified organisms is limited to those processes in which conditions are controlled so that the release of these organisms to the environment does not occur, so it is not possible to apply this strategy in soils.

The use of plants for decontamination purposes is called phytoremediation. Plants have unique ability to concentrate essential and nonessential elements from the soil through the roots (Eapen and D'Souza 2005). It may be applicable for the remediation of metal(loid)s, radionuclides, and organic pollutants, although in this chapter we are going to refer to metal(loid)s. The concept of phytoremediation was introduced by Chaney (1983), and comes from the Greek word *phyto*, meaning plant, and the word *remedium*, in Latin, meaning to correct. It is also known as botanoremediation, vegetative remediation, green remediation, or agroremediation (Khalid et al. 2017). It is a cost-effective, environmentally friendly, in situ applicable, and solar-driven remediation technology (Ali et al. 2013a). Phytoremediation is defined by the United Nations Environment Programme as the use of living green plants for in situ removal, degradation, and containment of contaminants in soils, surface waters, and groundwater.

The mechanisms of phytoremediation have been discussed in a considerable number of reviews (Agnello et al. 2014; Ali et al. 2013a; Baker and Brooks 1989; Gomes et al. 2016; Jan et al. 2016; Latif and Amin 2017; Lee 2013; Mani and Kumar 2014; Mench et al. 2010; Miransari 2011; Padmavathiamma and Li 2007; Prasad and Freitas 2003; Pulford and Watson 2003; Sheoran et al. 2016; Vamerali et al. 2010; Vangronsveld et al. 2009; Zhao and McGrath 2009). The main disadvantage is the longer time required compared to other technologies; it may be seasonal depending on the location, its applicability is limited for moderate pollutant concentration and should be located at low depth, pollutants have the potential to be transferred to another medium and/or food chain, and high concentrations of metal(loid)s can be toxic to plants. Seeds and fruits generally accumulate metal(loid)s at lower concentrations than leaves, shoots, or roots (Faessler et al. 2010). The ideal plants for phytoremediation purposes should show a high metal(loid) absorption capacity, be fast growing, yield large volumes of biomass, and be easy to harvest (Jan et al. 2016). Some methods to improve the phytoremediation potential of plants are the genetic engineering to obtain plants with genes that can confer accumulation abilities, the use of mobilizing agents to increase the availability of metal(loid)s, the combined use of microorganisms and plants, and the use of trees (Barbafieri et al. 2017; Clemens et al. 2002; Franchi et al. 2017, 2019; Gomes et al. 2016; Jan et al. 2016; Kacalkova et al. 2015; Meers et al. 2008; Miransari 2011; Pulford and Watson 2003; Sheoran et al. 2016). Plants used for phytoremediation purposes must be adequately treated to avoid the introduction of metal(loid)s into the trophic chain. In this sense, the use of the biomass as a source of energy (Asad et al. 2017; Jiang et al. 2015), or in the case of metal(loid)s of commercial interest, their recovery from the plants or phytomining, is extensively studied (Brooks et al. 1998; Chaney et al. 2018; Chaney and Baklanov 2017; Nkrumah et al. 2016; Robinson et al. 1999; Rosenkranz et al. 2018; Sheoran et al. 2009; Sinkala 2018; Tabasi et al. 2018). Metals like Ni, Co, and Au may have sufficient economic value in phytomining biomass to support commercial practices (Chaney et al. 2018).

Depending on the involved mechanism, phytoremediation can be phytoextraction (or phytoaccumulation), phytostabilization, phytofiltration (rhizofiltration), phytovolatilization, and phytodegradation (or phytotransformation) (Ali et al. 2013a; Eapen and D'Souza 2005; Vamerali et al. 2010). Phytoextraction (also known as phytoaccumulation, phytoabsorption, or phytosequestration) is the uptake of contaminants from soil or water by plant roots and their translocation into their harvestable tissues (Ali et al. 2013a; Eapen and D'Souza 2005; Gomes et al. 2016). Plants show differing morphophysiological responses to soil metal contamination. Most are sensitive to very low concentrations, others have developed tolerance, and some plant species (a reduced number) have the inherent ability to accumulate high concentrations of metal(loid)s in aboveground biomass without evident symptoms of toxicity and are known as hyperaccumulators (Baker and Brooks 1989; Baker et al. 2000; Reeves et al. 2018). The hyperaccumulators can accumulate hundreds to thousands of times more metal(loid)s than "normal" plants growing in the same environment (Lee 2013; Pulford and Watson 2003; Reeves et al. 2018; Sheoran et al. 2016). The majority of hyperaccumulators known to date are related to nickel. Reeves et al. (2018) created an updatable database of hyperaccumulators which, in April 2019, contained 721 hyperaccumulator species, and 532 were Ni hyperaccumulators. The families most strongly represented are the *Brassicaceae* and the *Phyllanthaceae* (Reeves et al. 2018). In general, hyperaccumulator plants do not produce high biomass limiting their application for bioremediation (Miransari 2011). The application of genetic engineering or adequate agronomy practices can increase biomass (Jan et al. 2016; Zhao and McGrath 2009). Due to the limitations of hyperaccumulator plants, the use of crops which produce high biomass and an important metal(loid) accumulation can be an interesting option for phytoremediation techniques.

Phytostabilization or phytoimmobilization is the use of plants for stabilization of metal(loid)s in contaminated soils, reducing their mobility through accumulation and/or sorption in the root, complexation, precipitation, or metal(loid) reduction in the rhizosphere (Ali et al. 2013a; Gomes et al. 2016). Phytostabilization strategies reduce the bioavailability of metal(loid)s to plants and other soil organisms, limiting their transfer to trophic chain, and decrease the potential leaching of metal(loid)s to surface and groundwater.

Phytofiltration is used for polluted water; it can be categorized into rhizofiltration (use of plant roots), blastofiltration (use of seedlings), or caulofiltration (use of excised plant shoots) (Mesjasz-Przybylowicz et al. 2004; Sarma 2011). In these processes, the contaminants are absorbed or adsorbed and thus their mobility and potential leaching to groundwater are minimized.

Phytovolatilization is the uptake of pollutants from soil by plants, their conversion to volatile form, and subsequent release into the atmosphere through the stomata (Ali et al. 2013a; Gomes et al. 2016). This technique can be used for organic pollutants and some metals like Hg and Se. However, its use is controversial because the pollutant is not completely removed; it is transferred from soil to atmosphere from where it can be redeposited (Ali et al. 2013a; Padmavathiamma and Li 2007).

Phytodegradation is referred for organic pollutants and it is based on the capacity of the plants to accumulate organic contaminants and degrade them through their metabolic activities (Ali et al. 2013a).

5 Barley and Wheat Plants in Phytoremediation Processes

Cereal crops have been used in phytoremediation experiments due to their high biomass, quick and easy growth, high adaptation capacity at different soil typologies and climatic conditions, and their considerable tolerance to metal(loid) pollution (Farrag et al. 2012). The biomass produced by these crops may also be utilized after harvest as a source of renewable energy (Naik et al. 2010). A lot of work has been done to evaluate the capacity of different cereals such as barley and wheat for phytoremediation processes (Table 2). Many of the studies have been performed under hydroponic conditions. These experiments are useful to elucidate physiological mechanisms of the plants such as detoxification and sequestration of metal(loid)s and are the base of further phytoremediation experiences. Nevertheless, the extrapolation of the plant behavior under controlled hydroponic conditions to soil conditions is often limited (Stojic et al. 2016). Thus, the comparison of different experiments performed to date is difficult. In this chapter we have focused on barley and wheat because of their wide distribution across different soil typologies and climatic conditions. In this sense, barley can grow in regions where other cereals such as maize and rice cannot grow well; barley extends in most areas with Mediterranean climate, in zones with continental and oceanic climate, and even into the arctic and subarctic. In addition, barley can grow near desert areas such as North Africa, due to its good resistance to dry heat (Zhou 2009). Barley and wheat belong to the similar order, family, and tribe and the main difference between these crops is that wheat is a *Triticum* genus cereal, and barley is a *Hordeum* genus crop. Furthermore, Ebbs and Kochian (1998) found that barley accumulated two to four times more Zn than what was observed in Indian mustard (*Brassica juncea*) in the presence of EDTA.

Table 2 collects and summarizes the main researches on tolerance and phytoremediation capacity of barley and wheat plants to metal(loid)s pollution. The studies have been organized by metal(loid)s, firstly the case of single pollution, and then those performed with a mixture of metal(loid)s. Barley and wheat plants show different responses depending on the metal(loid)s present in the soil since the mechanisms of uptake and the harmful effects differ. For the same element, differences can be observed between both of them and even between different varieties or

Table 2 Compilation of published studies on phytoremediation and tolerance of metal(loid)s using barley and wheat plants

Metal(loid)	Plant	Type of experiment, medium and grade of pollution	Time of exposure	Mobilizing or immobilizing agents	Rhizobacteria	Plant behavior	References
As	Barley (*Hordeum vulgare*) Minorimugi	Phytotron, hydroponic, 6.7–67 µM	21 days	–	–	As mainly concentrated in root, dose dependent At the highest doses (33–67 µM) the concentrations of P, K, Ca, Mg, Fe, Mn, Zn, and Cu were reduced	Shaibur et al. (2008)
As	Barley (*Hordeum vulgare*) Minorimugi	Phytotron, hydroponic, 6.7–67 µM	21 days	–	–	As was mainly accumulated in roots, increased with As concentration In general, accumulation of P and the cations showed negative relationship with As. Fe was specially affected by As; PS accumulation in roots did not change at 0 and 33.5 µM but decreased at 67 µM	Shaibur et al. (2009)
As	Barley (*Hordeum vulgare*) Minorimugi	Phytotron, hydroponic, 10 µM; ammonium phosphate (50–500 µM)	1–2 months, until nutrient deficiency symptoms were very apparent	–	–	Fe-plaque was visible in the roots under As-treated and P-depleted conditions. Reddish coloration in the roots was not detected in the absence of As and P; reddish coloration in the roots decreased with increasing P. As uptake decreased at increasing concentrations of P	Shaibur et al. (2013)

As, Cd, Pb (single and mixture)	Barley (*Hordeum vulgare*) KWS Bambina E	Glass chambers, pot; spiked soil with As, Cd, and/or Pb	Complete crop cycle (5 months)	–	–	Barley plants were most affected by As and the mixture As+Cd + Pb (100, 10, and 150 mg/kg); metal phytoextraction order root > straw ≥ leaves > grains. Only As-affected reflectance spectra of leaves	Rathod et al. (2015)
As	Barley (*Hordeum vulgare*) Pedrezuela; wheat (*Triticum aestivum*) Albares	Greenhouse, pot; spiked soil 40 and 80 mg/kg + compost from sewage sludge composted with pruning waste	From stage 20 of Zadoks scale to the end of crop cycle (6 months)	–	–	Growth reduction. The addition of compost induced an increase of As translocation to the aerial part in barley, but it did not affect As distribution in wheat plants	González et al. (2019)
Cd	Barley (*Hordeum vulgare*) CM72, wheat (*Triticum aestivum*) AgCs	Growth chamber, hydroponic, Cd and Fe: 3×10^{-5} and 0; 0 and 3×10^{-5}; 3×10^{-5} and 3×10^{-5}	18 days for wheat, 8 days for barley	Chelates: (a) PS from barley plants; (b) rhizoferrin, a fungal siderophore produced by *Rhizopus arrhizus*; (c) HEDTA	–	Cd uptake was not influenced by Fe stress. PS and HEDTA favored Cd absorption, although there was no significant uptake of Cd-PS complex by the plant roots	Shenker et al. (2001)
Cd	Five genotypes of wheat (*Triticum aestivum*)	Greenhouse, hydroponic, 1 mg/L	From seedlings with two leaves to maturity	–	–	Growth reduction, differences between genotypes	Zhang et al. (2002)

(continued)

Table 2 (continued)

Metal(loid)	Plant	Type of experiment, medium and grade of pollution	Time of exposure	Mobilizing or immobilizing agents	Rhizobacteria	Plant behavior	References
Cd	11 genotypes of barley (*Hordeum vulgare*)	Hydroponic, 0.1–1 µM	26 days	–	–	Different behaviors depending on the genotype. Wumaoliuling was the most affected	Wu and Zhang et al. (2002)
Cd	Two genotypes of two wheat species, *Triticum aestivum*, Thasos and Tjalve; *T. turgidum var. durum*, Topdur and Grandur	Growth chamber, hydroponic, 0.01, 1, and 3 µM		–	–	Durum wheats showed higher Cd accumulation than bread wheats. Phytochelatins did not differ between species or varieties	Stolt et al. (2003)
Cd	Two genotypes of barley (*Hordeum vulgare*), Tokak and Hamidiye	Hydroponic, 15–120 µM	12–60 h	–	–	Different behaviors depending on the genotype. Hamidiye was more sensitive to Cd than Tokak. Hamidiye developed higher leaf toxicity symptoms, decrease in growth, and antioxidant activity. Cd in root increased with exposure time	Tiryakioglu et al. (2006)

Cd	105 genotypes of barley (*Hordeum vulgare*)	Hydroponic, 20 μM	20 days	–	–	From 105 genotypes of barley, 10 were selected as Cd tolerant and 5 as Cd sensitive. Significant differences in Cd accumulation. Weisuobuzhi, highest accumulation; Jipi 1, lowest translocation; Weisuobuzhi and Jipi 1: highest tolerant genotypes; Dong 17 and Suyinmai 2: the most sensitive genotypes	Chen et al. (2008)
Cd, Pb (single)	Barley (*Hordeum vulgare*) Tselinnyi-5	Pot experiment, Cd 30 and 75 mg/kg or Pb 100 and 500 mg/kg	40 days	–	*Azospirillum lipoferum* 137, *Arthrobacter mysorens* 7, *Agrobacterium radiobacter* 10, and *Flavobacterium* sp. L30	Cd: rhizobacteria improved the growth of plants and decreases the accumulation of Cd due to its immobilization in the rhizosphere and/or auxin production by rhizobacteria Pb: rhizobacteria improved the growth of plants and decreased Pb uptake depending on the strain and the Pb concentration Rhizobacteria mitigated toxic effects of Cd and Pb	Belimov et al. (2004)

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Table 2 (continued)

Metal(loid)	Plant	Type of experiment, medium and grade of pollution	Time of exposure	Mobilizing or immobilizing agents	Rhizobacteria	Plant behavior	References
Cd, Pb (single)	Barley (*Hordeum vulgare*) Tselinnyi-5	Field conditions	Complete vegetative cycle	–	Commercial preparations of *Azospirillum lipoferum* 137, *Arthrobacter mysorens* 7, *Agrobacterium radiobacter* 10, and *Flavobacterium* sp. L30	Rhizobacteria improved the growth of barley plants and decreased the accumulation of Cd and Pb depending on their concentration in soil and the strain. Rhizobacteria mitigated toxic effects of Cd and Pb	Belimov et al. (2004)
Cd, Cr, Zn (single)	Four genotypes of barley (*Hordeum vulgare*) Pedrezuela, CB502, Reinette, Plaisant	Greenhouse, pots with soil irrigated with 10–40 mM Cd, 1–3 mM Cr, or 50–250 mM Zn	From stage 20 of Zadoks scale to the end of crop cycle (5 months)	–	–	Metal toxicity and metal accumulation depended on genotype. Cd and Zn induced toxicity only at the highest doses, and it was much lower than Cr	González and Lobo (2013)
Cd	Two genotypes of durum wheat (*Triticum durum*), Creso and Svevo	Pots placed outdoor, 1.5, 3, and 4.5 mg Cd/kg	144 days for Svevo; 151 days for Creso	–	–	Plant biomass was not affected; Cd increased in grain depending on the genotype (>0.2 mg/kg)	Arduini et al. (2014)

Cd	Wheat (*Triticum aestivum*) Lasani-(2008)	Wirehouse, soil contaminated due to irrigation with raw city effluents	130 days	Biochar (1.5, 3, and 5% w/w)	–	Biochar improved the growth of the wheat plants, photosynthetic activity, and gas exchange in leaves. Biochar decreased Cd concentration in plants	Abbas et al. (2017)
Cd, Cr (single)	Two genotypes of barley (*Hordeum vulgare*) Pedrezuela and CB502	Greenhouse; pots with soil irrigated with 30–60 mM Cd or 0.2–1.5 mM Cr	From stage 20 of Zadoks scale to the end of crop cycle (5 months)	–	–	Cr was more toxic than Cd for both varieties. Different behaviors depending on the variety Cd: no effect on chlorophyll content. Higher accumulation in root than shoot, not dose dependent. Pedrezuela higher tolerance and translocation Cr: strong effect on the growth and physiology of plants. Higher accumulation in root than shoot, dose dependent. CB502 higher tolerance, higher root concentration than Pedrezuela	González et al. (2015)
Cd, Cr (single)	Two genotypes of barley (*Hordeum vulgare*), Pedrezuela and CB502, and one of wheat (*Triticum aestivum*) Albares	Greenhouse; pots with soil irrigated with 40–60 mM Cd or 0.5–1.5 mM Cr	From stage 23 of Zadoks scale to the end of crop cycle (5 months)	–	–	Cr was more toxic than Cd. Cd did not affect chlorophyll content, fluorescence values, or biomass of the three cultivars whereas these parameters decreased with Cr increasing metal concentrations. Barley more effective for phytoremediation of Cd- or Cr-polluted sites	González et al. (2017a)

(continued)

Table 2 (continued)

Metal(loid)	Plant	Type of experiment, medium and grade of pollution	Time of exposure	Mobilizing or immobilizing agents	Rhizobacteria	Plant behavior	References
Cd, Ni, Pb (single)	Wheat (*Triticum aestivum*) PBW-343	Growth chamber, hydroponic conditions (iron deficient and iron sufficient), 2.5, 5, and 10 μM of Cd, Ni, or Pb	3 days	–	–	Cd: the increase of Cd concentration was dose dependent Pb: higher accumulation at 2.5 and 5 μM than at 10 μM Ni: similar accumulation at the three doses In iron-deficient conditions, the PS release was not inhibited by the presence of Cd, Ni, Pb; Cd and Pb induced higher PS release than Ni	Gupta and Singh (2017)
Cr, Zn (single)	Barley (*Hordeum vulgare*) Pedrezuela, wheat (*Triticum aestivum*) Albares	Greenhouse; pots with soil 10 and 22 mg Cr/kg or (3000) and (6000) mg Zn/kg	From stage 23 of Zadoks scale to the end of crop cycle (5 months)	–	–	Wheat was more tolerant to Zn and barley to Cr. Barley showed higher translocation of Zn	González et al. (2017b)
Cr	Wheat (*Triticum aestivum*), compared with 6 other crops	Wirehouse, soil contaminated with tannery effluents	4 months	EDTA, 1, 5, and 10 mmol/kg	–	EDTA addition increased Cr uptake. No strong effect on wheat biomass	Firdaus and Tahira (2010)

Cr	Barley (*Hordeum vulgare*), Hua 30	Greenhouse, hydroponic, 75 and 100 µM Cr; three forms of N: $(NH_4)_2SO_4$, urea, and $Ca(NO_3)_2$	30 days	–	–	Plants fed with $Ca(NO_3)_2$ accumulated the lowest Cr and the highest N content in all plant tissues; the former showed more oxidative damage and growth reduction	Ali et al. (2013b)
Cr	Wheat (*Triticum aestivum*) UP262	Growth chamber, hydroponic, Cr(VI) 5–100 µM	7 days	EDTA, citric acid, $ZnSO_4$ (50 µM)	–	Chlorophyll content decreased with Cr concentration. Chelates induced lowest growth rate, especially with citric acid; the maximum Cr uptake (in root and shoot) was with citric acid chelate	Das et al. (2014)
Cr	Wheat (*Triticum aestivum*) Lasani (2008)	Pot experiment in a botanical garden with sand or soil, irrigated with Cr 0.25, 0.5 mM. Foliar application of FA (1.5 mg/L)	4 months	–	–	Plant grown in sand accumulated more Cr than those grown in soil. Cr increased in all the parts of the plant without FA, in both soil and sand. The addition of FA decreased Cr toxicity, decreasing its uptake and translocation, increasing biomass, chlorophyll content, and antioxidant defense	Ali et al. (2015)
Cu	Barley (*Hordeum vulgare*) compared with other 6 crops	Pots at field conditions, Cu 200, 400, 600 mg/kg	Complete vegetative cycle (repeated 2 years)	–	–	Similar Cu accumulation in both years. Distribution: roots >> grain > leaves and stems. Leaves and stems showed higher biomass at 400 and 600 mg Cu/kg; no effect on root biomass	Napoli et al. (2019)

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Table 2 (continued)

Metal(loid)	Plant	Type of experiment, medium and grade of pollution	Time of exposure	Mobilizing or immobilizing agents	Rhizobacteria	Plant behavior	References
Cu, Pb, Zn (single)	Barley (*Hordeum vulgare*), wheat (*Triticum aestivum*)	Hydroponic, 1, 5, and 10 μM of Cu, Pb, or Zn	3 days	–	–	The effect on seed germination and seedling growth was ordered as Cu > Zn > Pb. Inhibitory effects higher in wheat than barley	Mahmood et al. (2007)
Hg	Barley (*Hordeum vulgare*), compared with other 3 crops	Three experiments: (a) Hydroponic, vermiculite, and perlite spiked with Hg (2 mg/pot) (b) Polluted soil (8 μg/g) + perlite (2:1) (c) Polluted soil (8 μg/g) + perlite (2:1) + spiked Hg (2 mg/pot)	150 days	–	–	Different results under hydroponic and soil conditions. Spiking the soil increased Hg availability and Hg uptake	Rodriguez et al. (2007)
Ni	Barley (*Hordeum vulgaris*), compared with other 6 crops	Pot experiment, soil spiked with 150, 300, and 600 mg Ni/kg	Complete vegetative cycle (repeated 2 years)	–	–	Differences between both years. At the lowest Ni concentration, barley biomass increased	Giordani et al. (2005)
Pb	Wheat (*Triticum aestivum*) Atlas 66	Greenhouse, (10,600) mg Pb/kg, 15 mg Cd/kg, 233 mg Cu/kg, 501 mg Zn/kg	31 days, 7 and 14 days after EDTA first addition	EDTA, 3 mmol/kg	–	EDTA addition decreased wheat biomass. Pb concentration increased in root and shoot	Shen et al. (2002)

Pb	Barley (*Hordeum vulgare*) Arminda	Mixture of peat moss, forest soil, silt and perlite (3:2:1:1 v/v) amended with powdered Pb slag (10, 20, 30%)	Since emergence to flowering stage	–	AMF consortium	AMF consortium had positive impact on barley plant development and favored the extraction of Pb	Arias et al. (2015)
Pb	Wheat (*Triticum aestivum*) Uqab-(2000)	Wirehouse, spiked soil (500 Pb mg/kg)	–	EDTA (4 mmol/kg) and elemental S (200 mmol/kg), as a single dose or split doses	–	The addition of S alone or with EDTA improved photosynthetic activity; EDTA-split + S was the most effective. EDTA-split + S produced the highest increase of Pb in shoot whereas S alone produced the least Pb accumulation	Saifullah and Qadir (2009)
Pb	Wheat (*Triticum aestivum*) Auqab-(2000)	Wirehouse, spiked soil (500 mg Pb/kg)	5 months	EDTA (2, 4, 8 mmol/kg), citric acid (10, 20, 30 mmol/kg), S (50, 100, 200 mmol/kg)	–	Citric acid increased the phytoextraction of Pb more than EDTA and S; S was ineffective for enhancing Pb in shoot	Saifullah et al. (2010b)
Pb	Two genotypes of wheat (*Triticum aestivum*) Auqab-(2000) and Inqalab-91	Wirehouse, spiked soil (500 mg Pb/kg)	–	EDTA, 2, 4, 8 mmol/kg	–	Different behaviors depending on variety, Inqalab was more tolerant to Pb than Auqab. EDTA favored with Pb uptake and translocation to shoots, especially in Auqab	Saifullah et al. (2010a)

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Table 2 (continued)

Metal(loid)	Plant	Type of experiment, medium and grade of pollution	Time of exposure	Mobilizing or immobilizing agents	Rhizobacteria	Plant behavior	References
Pb	Wheat (*Triticum aestivum*) Auqab-(2000)	Wirehouse, spiked soil (500 mg Pb/kg), two types of soils	4 months	EDTA (2, 4, 8 mmol/kg), S (100, 200 mmol/kg)	–	Differences between the two types of soils. Application of EDTA resulted in greater accumulation of Pb compared with S, especially in the loamy sand soil	Saifullah et al. (2010c)
Cd, Pb	Wheat (*Triticum aestivum*) Inqlab-91	Pot irrigated with Pb and Cd solution (260 mg/pot)	Complete vegetative cycle	Farm manure, gypsum, $CaCO_3$,	–	Organic and inorganic amendments decreased Pb and Cd uptake, especially in the sandy clay loam soil	Ahmad et al. (2011)
Pb	Wheat (*Triticum aestivum*) Shafaq-(2006)	Greenhouse, spiked soil (50, 100 mg Pb/kg)	Complete vegetative cycle	S (150, 300 mmol/kg)	–	S improved photosynthetic activity, transpiration rates, biomass and Pb accumulation in roots, shoot, and grain	Saifullah et al. (2016)
Sr	4 genotypes of barley (*Hordeum vulgare*), 6 of wheat (*Triticum aestivum*)	Greenhouse, hydroponic, 100 and 500 mg/kg Sr	20 days	–	–	In general, no significant effect on shoot biomass, and no apparent toxic effect on the accumulation of shoot. Shoots of barley showed the highest concentration and the lowest was in wheat	Qi et al. (2015)
Zn	Barley (*Hordeum vulgare*) compared with three other crops	Growth chamber, pot experiment 881 mg/kg Zn	5 weeks, 5 days after EDTA treatment	EDTA 0–3.4 mmol/kg	–	The complex Zn-EDTA was taken up via an apoplastic pathway (passive extracellular transport into the xylem)	Collins et al. (2002)

Cd, Fe, Mn, Ni, Pb, Zn (mixture)	Barley (*Hordeum vulgare*) Weskan	Column experiment, soil + biosolids (120 kg N/ha, approximately 1–2 mm layer)	28 days; 4 days after EDTA addition	EDTA, 0.5 g/kg	–	EDTA favored the metal leachability, except for Cu. The concentration of Cd, Ni, and Pb decreased in the top 30 cm in soil with EDTA. Plants stopped growing even 48 h after EDTA treatment. Concentration of metals in shoot increased with EDTA	Madrid et al. (2003)
As, Cd, Cu, Pb, Tl, and Zn (mixture)	Barley (*Hordeum distichum*) Trebon, compared with other 3 crops	Field experiment in three different locations flooded by the Aznalcóllar spill	Complete vegetative cycle	–	–	The most polluted areas induced a reduction of biomass and the highest accumulation of metal(loid) s	Soriano and Fereres (2003)
Cd, Pb (mixture)	Barley (*Hordeum vulgare*) Tara, compared with other crops	Field conditions, Cd 1.81 mg/kg, Pb 22 mg/kg	Complete vegetative cycle	–	–	Cd and Pb were accumulated mainly roots >> straw > grain. Pb accumulation in barley roots was higher than the other crops	Sekara et al. (2005a)
Cu, Zn (mixture)	Barley (*Hordeum vulgare*) Tara, compared with other 8 crops	Field experiment (187 mg Cu/kg, 12.5 mg Zn/kg)	Complete vegetative cycle	–	–	Low translocation of Cu and Zn; Cu was 6.5 more concentrated in roots than aboveground tissues	Sekara et al. (2005b)
Cd, Cu, Ni, Pb, Zn (single and mixture)	Barley (*Hordeum vulgare*) Weskan	Greenhouse, spiked soil 100 and 500 mg/kg for Cu and Zn; 50 and 250 mg/kg for Cd, Ni and Pb	41 days; 3, 6, and 10 days after EDTA addition	EDTA, 0.5 g/kg	–	EDTA favored the metal uptake both for single- and multi-metal contamination. Roots had a higher concentration of the added metal than the soil, in EDTA treatments	Madrid et al. (2008)

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Table 2 (continued)

Metal(loid)	Plant	Type of experiment, medium and grade of pollution	Time of exposure	Mobilizing or immobilizing agents	Rhizobacteria	Plant behavior	References
Cd, Cu, Fe, Mn, Ni, Pb, Zn (mixture)	Barley (*Hordeum vulgare*) Tambar 501	Column experiment, soil + sewage sludge + saltwater irrigation	23 days	EDTA 0.5 g/kg	–	EDTA increased metal solubility. Irrigation with NaCl solution in EDTA-treated soils also increased metal availability and barley plants died (only 7 out of the 30 plants survived at harvest); Fe, Mn, and Zn increased in shoots; Fe, Ni, and Pb in roots	Wahla and Kirkham (2008)
Cu, Pb, Zn (mixture)	Barley (*Hordeum vulgare*)	Pots at field conditions with soil from a mine	8 weeks	–	–	Metal concentration followed the order Zn > Pb > Cu. Higher translocation of Cu and Zn from roots to shoots than Pb	Ruiz et al. (2009)
Cd, Cr, Cu, Ni, Pb, Zn (mixture)	Barley (*Hordeum vulgare*), Mattina; wheat (*Triticum durum*) Simeto	Field conditions, 0.78–1.19 mg Cd/kg, 55–1792 mg Cr/kg, 31–321 mg Cu/kg, 47–812 mg Pb/kg, 36–54 mg Ni/kg, 111–924 mg Zn/kg	160 days	–	*Bacillus licheniformis* BLMB1	Higher metal accumulation in roots, Zn > Cr > Cu > Pb > Ni > Cd. Application of *B. licheniformis* increased accumulation of metals in roots, and translocation of Cd, Cr, and Pb for barley	Brunetti et al. (2012)
Cr, Cu, Pb, Zn (mixture)	Barley (*Hordeum vulgare*), wheat (*Triticum durum*)	Field conditions, 55–1792 mg Cr/kg, 31–321 mg Cu/kg, 47–812 mg Pb/kg, 111–924 mg Zn/kg	160 days	–	*Bacillus licheniformis*	Better plant develops in polluted soils. The effect of Bacillus inoculation depended on soil properties	Farrag et al. (2012)

Cd, Cr, Cu, Ni, Pb Zn (mixture)	Barley (*Hordeum vulgare*)	Greenhouse using 36 different soils amended with two sewage sludges with different levels of metal(loid)s	8 weeks	–	–	The transfer of metals to plant depended on soil properties and metal, observing similar patterns in soils amended with the same sewage sludge	Martin Soriano-Disla et al. (2014)
As, Cd, Cu, Fe, Hg, Mn, Ni, Pb and Zn (mixture)	Barley (*Hordeum vulgare*) and wheat (*Triticum aestivum)*	Hydroponic and soil conditions. Three types of irrigation: artificial rainwater solution, metal(loid) irrigation solution, metal irrigation solution + liquid manure containing NH_4NO_3, H_3PO_4, KOH	20 days	–	–	Barley and wheat showed different accumulation of metal(loid)s. Higher concentrations of As, Cd, Hg, Ni, and Pb in both plants irrigated with metal(loid) solution. The presence of soil decreased Hg uptake. The addition of liquid manure reduced phytoavailability of As, Cu, Fe, and Pb in pot experiment and As, Pb, and Zn in hydroponic experiment	Stojic et al. (2016)
Cr, Cu, Ni, Zn (mixture)	17 genotypes of barley (*Hordeum vulgare*)	Pot experiment, soil polluted with Cr, Cu, Ni, Zn (due to irrigation with water polluted with sewage sludge, industrial effluents, household activities, and agricultural practices)	Complete vegetative cycle	–	–	Differences between genotypes, six showed higher accumulation of these metals in grain and leaves	Ali et al. (2017)

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Table 2 (continued)

Metal(loid)	Plant	Type of experiment, medium and grade of pollution	Time of exposure	Mobilizing or immobilizing agents	Rhizobacteria	Plant behavior	References
Cd, Co, Cr, Cu, Mn, Ni, Pb (mixture)	Wheat (*Triticum aestivum*)	Field conditions, saline sodic field	57 days after inoculation	–	*Bacillus cereus*, *Pseudomonas moraviensis,* using a biofertilizer as carrier (maize straw and sugarcane husk)	Rhizobacteria showed tolerance to saline conditions and to metals, especially Cd and Mn. PGPR in the form of biofertilizer was the most effective; it reduced the accumulation of metals in plant and enhanced their translocation	Ul Hassan et al. (2017)

TF translocation factor, *PS* phytosiderophores, *AMF* arbuscular mycorrhizal fungi, *FA* fulvic acid, *PGPR* plant growth-promoting rhizobacteria genotypes belonging to the same species. Soil properties also affect metal(loid) toxicity to plants (Ahmad et al. 2011). In general, high concentrations of metal(loid)s in soil may induce a reduction in cereal biomass and chlorophyll content, affecting physiological parameters and antioxidant activity and producing visual toxicity symptoms. In general, grain accumulates lower quantity of metal(loid)s than the roots, shoots, and leaves (Abbas et al. 2017; González et al. 2012, 2013, 2019, 2015, 2017a, b; Rathod et al. 2015; Saifullah et al. 2016).

Differences between cultivars or genotypes belonging to the same species are often detected (Ali et al. 2017; Arduini et al. 2014; Brunetti et al. 2012; Chen et al. 2008; Farrag et al. 2012; González et al. 2013, 2015, 2017a, b; Mahmood et al. 2007; Saifullah et al. 2010a, b; Shenker et al. 2001; Stojic et al. 2016; Stolt et al. 2003; Tiryakioglu et al. 2006; Wu and Zhang 2002; Zhang et al. 2002). Thus, the tolerance and the phytoremediation capacity should be taken into account before choosing the most suitable cultivar for a phytoremediation process. In this sense, Stolt et al. (2003) concluded that durum wheat accumulated higher concentrations of Cd than bread wheat. Tiryakioglu et al. (2006) performed a hydroponic study using two genotypes of barley (Hamidiye and Tokak) exposed to Cd for 60 h. Although both genotypes showed a similar accumulation of Cd in roots, shoots, and leaves, Hamidiye presented the most severe toxicity symptoms including chlorosis and reddish-brown discoloration of leaves and browning of roots, decrease in biomass, as well as a higher antioxidant response against reactive oxygen species (ROS). Qi et al. (2015) compared the uptake and distribution of Sr in 26 cultivars of wheat, barley, and oat grown at hydroponic conditions. Overall, the plants did not show toxic effects due to the Sr and wheat plants had the lowest Sr accumulation. González and Lobo (2013) studied the effect of different concentrations of Cd, Cr, and Zn on the development of four barley cultivars (CB502, Reinette, Pedrezuela, and Plaisant) during the complete crop cycle. The results showed that Cr induced more toxicity than Cd and Zn. The four barley cultivars exhibited a different behavior, CB502 and Reinette were the most tolerant, CB502 accumulated higher concentration of Cr, Plaisant accumulated higher concentration of Zn, and Reinette accumulated higher concentration of Cd. Subsequently, González et al. (2015) studied the tolerance of two different cultivars of barley, Pedrezuela and CB502, to increasing concentrations of Cd and Cr incorporated to the soil by irrigation. Both cultivars showed higher tolerance to Cd than to Cr, and CB502 had a significant higher accumulation of Cd and Cr in roots. In a later study, González et al. (2017b) compared the tolerance of a variety of barley (Pedrezuela) and another from wheat (Albares) to Cr and Zn in a greenhouse experiment during all the crop cycle. The authors found that wheat was more tolerant to Zn and barley was more tolerant to Cr. Although barley showed less tolerance to Zn, its higher TF (transfer factor) and biomass make this plant adequate for remediation process. Regarding Cr, barley mainly accumulated it in root, and showed a greater capacity to assimilate nutrients under Cr-stress conditions. These results highlighted the potential capacity of Albares wheat to be used in soils contaminated by Zn and of Pedrezuela barley for use in Cr- and Zn-contaminated soils when the metal concentrations are similar or lower than those used in that experiment (Zn 3000–6000 mg/kg; Cr 10–22 mg/kg).

These studies showed barley more resistant to metal(loid) pollution than wheat (González et al. 2017a, b, 2019; Mahmood et al. 2007; Qi et al. 2015). In this sense, Mahmood et al. (2007) in a study at hydroponic conditions with Cu, Zn, or Pb found more pronounced inhibitory effects on wheat than on barley plants.

Different plant behavior has been detected in the case of single- or multi-metal(loid) contamination due to antagonism or synergism interactions among metal(loid)s (Ahmad et al. 2011; Ali et al. 2017; Rathod et al. 2015). In this sense, Rathod et al. (2015) studied the impact of polluted soils with As, Cd, and Pb, as single or mixture on the growth of barley plants, and they found that As and the multi-metal(loid) contamination induced higher toxicity in barley plants. Ali et al. (2017) found a different behavior between 17 genotypes of barley grown in a multi-polluted soil with Cr, Cu, Ni, and Zn. The authors concluded that none of the genotypes studied showed the best results for the accumulation of all the metals but differences depending on genotypes and metals were detected, and 6 out of 17 genotypes showed higher accumulation of these metals in grains and leaves. They proposed the use of a mixture of different genotypes of barley to remediate this kind of polluted sites.

As previously commented, the behavior of the plants to metal(loid) pollution is different under hydroponic or soil conditions. Overall, plants absorb a greater amount of metal(loid)s under hydroponic conditions due to their higher bioavailability. In soils, the toxicity of metal(loid)s depends on their availability and it is conditioned by metal(loid) characteristics, soil properties (pH, texture, organic matter, cation exchange capacity, content of oxides and hydroxides of Al, Mn, and other metals), and type of plant (Ahmad et al. 2011; Saifullah et al. 2010c). Cationic metals, such as Cd, Cu, Pb, Ni, and Zn, are more available in acidic soils, while As (both As(III) and As(V)) are stronger adsorbed to oxides of Mn, Fe, and Al in acid soils. It has been shown that inorganic As is more toxic in sandy soil than in clay soils due to the fact that the latter contains more iron oxyhydroxides which strongly adsorb As. Ahmad et al. (2011) found a different absorption of Cd and Pb in wheat plants grown in texturally different soils (higher in a sandy loam soil compared with a sandy clay loam one). Stojic et al. (2016) compared the uptake of metal(loid)s (As, Cd, Cu, Fe, Hg, Mn, Ni, Pb, and Zn) by wheat plants under hydroponic (in Petri dishes) and soil conditions for 20 days. The experiment evaluated three types of irrigation: (a) artificial rainwater; (b) metal(loid) solution containing 0.1 mmol/L of As, Cd, Cu, Fe, Hg, Mn, Ni, Pb, and Zn; and (c) equal amount of metal(loid)s and liquid manure solution containing NH_4NO_3, H_3PO_4, and KOH. The experiment in Petri dishes was also performed for barley plants. Under hydroponic conditions, both plants showed a higher uptake of metal(loid)s in the case of irrigation with metal(loid) solution higher than that with metal(loid)s and liquid manure solution, with the exception of Hg, Zn, and Mn. Barley and wheat showed a different behavior, barley accumulated nearly two times lower concentration of As, whereas the application of liquid manure induced the equalization of the As accumulation by both plants. Wheat plants grown in pot showed a significant lower accumulation of As, Cd, Hg, Ni, Pb, and Zn compared with those grown in Petri dishes. In the same way, Rodriguez et al. (2007) compared the translocation of Hg in four crops (barley, white lupine, lentil, and chickpea), using hydroponic conditions and polluted soil from a mercury mine (alone and spiked with Hg), and they found a higher uptake of Hg under hydroponic conditions compared with soil conditions. In the case of Hg-spiked soil, the Hg accumulation for barley increased by a factor of 3.5 with respect to the non-spiked soil due to a higher availability of Hg. This result evidences the importance of metal(loid) availability for the phytoremediation process. Ali et al. (2015) performed an experiment with wheat plants grown in pots with soil or sand, irrigated with increasing concentration of Cr solution. The results showed a significant higher accumulation in plants grown in sand compared with those grown in soil. In this experiment, it was observed that the foliar application of fulvic acid on wheat leaves reduced Cr toxicity and improved the development of the plants, but it reduced the accumulation and translocation of Cr. Thus, the foliar application of fulvic acid would not be advisable to encourage phytoremediation strategies.

The sensitivity of barley and wheat plants strongly depends on the metal(loid). There are some comparative studies which evaluate the impact of different metal(loid)s at the same experimental conditions. According to them, the toxicity of metal(loid)s for barley and wheat could be ordered as Zn < Cd and Pb < As < Cr (González et al. 2013, 2015, 2017a, b; Madrid et al. 2008; Rathod et al. 2015). Rathod et al. (2015) studied the changes in leaf reflectance spectra (350–2500 nm) due to metal phytoextraction into barley plants grown in metal(loid)-spiked soils with As, Cd, and/or Pb and only detected changes in As treatment.

The inoculation of bacteria in the roots of the plants, rhizobacteria, to stimulate the phytoremediation process has shown different results. In this sense, Belimov et al. (2004) investigated the effect of the inoculation rhizobacteria on barley plants cultivated in soil contaminated with Cd and Pb and they found that the presence of rhizobacteria improved the growth of barley plants, with an increase of the uptake of nutrients and a decrease in metal accumulation. In this case, the addition of rhizobacteria improved the resistance of the plants to the stress induced by the presence of Cd and Pb but did not stimulate the phytoextraction capacity of the barley plants. In contrast, the application of *Bacillus licheniformis* strain BLMB1 to wheat and barley plants at field conditions improved the accumulation of Cr, Cd, Cu, Pb, and Zn in roots of wheat and barley, and increased Cd, Cr, and Pb contents in the shoots of barley (Brunetti et al. 2012). Arias et al. (2015) found that the application of an arbuscular mycorrhizal fungi consortium had a positive effect on barley plants encouraging the Pb extraction. In the same way, Ul Hassan et al. (2017) concluded that the inoculation of the roots of wheat plants with *Bacillus cereus* and *Pseudomonas moraviensis*, using a biofertilizer as carrier (maize straw and sugarcane husk), increased the tolerance of wheat to saline conditions and metal pollution, increasing the translocation of metals. González et al. (2019) studied the combined effect of compost and microorganisms from rhizosphere on the tolerance of barley and wheat plants to soils polluted with As. The results showed a different behavior between barley and wheat; that is, the addition of compost induced an increase of As translocation to the aerial part in barley plants but not in the wheat ones. The different bacterial communities' structure found for each species suggested that specific rhizobacteria of barley may have increased As bioavailability, and would therefore enhance its translocation to aerial parts.

Mobilizing agents, such as EDTA, citric acid or other chelates, and elemental sulfur, have been used in order to improve the phytoremediation effectives of barley and wheat plants (Ahmad et al. 2011; Collins et al. 2002; Das et al. 2014; Firdaus and Tahira 2010; Madrid et al. 2003, 2008; Saifullah and Qadir 2009; Saifullah et al. 2010a, b, c, 2016; Shen et al. 2002; Shenker et al. 2001; Wahla and Kirkham 2008). In this sense, Saifullah et al. (2010b) compared the effectiveness of EDTA, citric acid, and elemental sulfur to enhance the Pb-phytoremediative ability of wheat plants. At the experimental conditions, the authors found that EDTA was more effective than citric acid and elemental sulfur in solubilizing Pb from the soil, although citric acid (at 30 mmol/kg) was the best agent for enhanced phytoextraction of Pb using wheat plants. In a later study, Saifullah et al. (2016) observed that the addition of elemental sulfur improved the photosynthetic activity, transpiration rates, and biomass of wheat plants grown in Pb-polluted soils, as well as significantly increased the accumulation of Pb in roots, shoots, and grains. Thus, the authors concluded that by mitigating the toxic effect of Pb and improving wheat growth, sulfur enhances Pb accumulation by the aboveground plant parts and hence the phytoextraction capacity of wheat. The addition of chelates or other agents which increase metal(loid) availability has to be controlled because of the increase of risk of metal(loid) leachability to groundwaters and the impact of these compounds on soil properties and soil biota.

In contrast, the addition of organic matters or other amendments can decrease metal(loid) availability in soil reducing the accumulation of metal(loid)s in the different parts of the plants. In this sense, Hamid et al. (2019) performed a field-scale experiment with rice and wheat in a soil with Cd (0.83 mg/kg) and Pb (54.39 mg/kg), and evaluated the effect of the addition of 12 different amendments (single or mixtures of lime, biochar, Fe-biochar, hydroxyapatite, sepiolite, zeolite, organic manure, wood powder, Ca-Mg-P fertilizer) on metal availability and their absorption by both plants. All the tested amendments reduced Cd and Pb availability and their uptake by rice and wheat plants. In the same way, Ahmad et al. (2011) concluded that the application of farm manure to a Cd- and Pb-polluted soil significantly decreased the metal uptake by wheat plants. Abbas et al. (2017) observed that the application of rice straw biochar to a Cd-polluted soil improved wheat plant growth and gas exchange in leaves and decreased the Cd accumulation in root, shoot, and grain of wheat plants. Thus, in these cases, the amendments did not favor the phytoremediation process but reduced the risk of metal transfer to the food chain.

Overall, the decontamination strategies based on phytoremediation using barley or wheat plants, or even with hyperaccumulator plants, are very slow. As previously commented, different strategies, including the inoculation of adequate rhizobacteria or the addition of mobilizing agents, can be applied to increase the phytoremediation effectiveness of the barley and wheat plants. In some cases, the calculations indicate that it would take many years, decades, or even centuries to decontaminate a polluted soil. However, it should be noted that plants take up the most available fraction of metal(loid)s reducing their potential leachability as well as other risks associated to the presence of metal(loid)s in the soil. Specific studies at lab scale are strongly necessary before performing a phytoremediation project. Wan et al. (2016) performed a 2-year phytoremediation project for soil contaminated with As, Cd, and Pb with a total area of 19.5 ha, and reduced their concentration below China National Standards. They concluded that the total cost of the phytoremediation process was US\$37.7/m^3, including infrastructure, land preparation, irrigation, labor, cost of incineration, materials, and other direct and indirect costs. The total cost of this phytoremediation project was lower than that of other remediation technologies such as physicochemical strategies (Table 1), and the benefits of the phytoremediation strategy due to the planting of cash crops were expected to offset the costs in less than 7 years. In addition, it is necessary to take into account that the phytoremediation strategies are environmental friendly and plants contribute to minimize the soil erosion avoiding the loss of soil, and improve the soil fertility; the obtained biomass can be used for ethanol production or can be burnt for heat or electricity production and the phytomining of specific meta(loid)s can be considered. Thus, depending on the effectiveness of the plants for the metal(loid) extraction or stabilization, phytoremediation can be considered as a remediation strategy or as a strategy for reducing the risk associated to a moderate case of pollution. In this sense, the use of wheat or barley in phytomanagement process should be performed after pilot-scale assays to determine the more adequate species for each polluted site.

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Role of Beneficial Microbes in the Molecular Phytotoxicity of Heavy Metals

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1 Introduction

Large areas of soil have been polluted by heavy metal elements because of excessive mining, agriculture and industrial activities, which cause serious damages to soil ecosystems. These heavy metals when entering plants produce various negative effects like reduced growth, photosynthesis, tilted growth and generation of oxidative stress. Various negative effects are also produced in humans when these metal-contaminated plants are consumed. In order to overcome these effects of heavy metals, a large number of conventional and novel methods have been proposed to remediate heavy metal-contaminated soils, which include chemical precipitation, electrochemical treatment, ion exchange, oxidation/reduction, etc. (Zou et al. 2016). But these technologies have been limited in practical application, due to disadvantages such as high cost, complexity and secondary pollution. Therefore, the application of in situ chemical immobilisation and phytoremediation has been suggested as an eco-friendly and cost-effective method for the treatment of heavy metal-polluted soils (Liang et al. 2016). A number of metal-hyperaccumulating plants have already been identified as effective for the remediation of metal-polluted soil. Various methods have been successfully used to generate plants able to grow in adverse environmental conditions and accumulate or transfer a number of metals. In order to increase the efficiency of phytoremediation, microbial interactions with the plants have been explored for their use in HM removal from contaminated environments. Microorganisms can affect metal solubility in soil and their availability to plants. Improvement in the interactions between plants and microorganisms can promote the plant biomass production and tolerance of the plants to HM and is considered to be an important component of phytoremediation technologies. In this chapter we have discussed about the use of hyperaccumulating plants in the remediation of metal-contaminated soil and how their efficiency could be increased with the application of various microbes. Mechanisms adopted by the microbes and various plant-microbial interactions are also discussed in detail that are useful in the process of bioremediation, a green technology for the remediation of metal pollution.

2 Sources of Heavy Metals

Due to anthropogenic activities, heavy metal pollution has emerged as one of the major threats to all life forms. Heavy metals are naturally occurring elements having density higher than 5 g cm^{-3}. In today's era of global modernisation, industrial development, urbanisation and other anthropogenic activities are the need of the hour and are associated with huge application of heavy metals (Tochounwou et al. 2012; Oves et al. 2016). Fifty-three of the ninety naturally occurring elements are heavy metals but all of these are not biologically important. At permissible amount their use in industrial, domestic, agricultural, medical and technological areas has been increased at an exponential rate, which eventually led to their wide prevalence in the environment, thereby raising the concerns over their potential effects on human health and the environment. On the contrary metals such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se) and zinc (Zn) are essential nutrients as they play a significant role in different biochemical and physiological activities and their availability below required amount causes inadequate deficiency diseases or syndromes. However metals such as aluminium (Al), antinomy (Sb), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), gallium (Ga), germanium (Ge), gold (Au), indium (In), lead (Pb), lithium (Li), mercury (Hg), nickel (Ni), platinum (Pt), silver (Ag), strontium (Sr), tellurium (Te), thallium (Tl), tin (Sn), titanium (Ti), vanadium (V) and uranium (U) have not considered to be biologically important and thus are known as non-essential metals (Tochounwou et al. 2012; Chia et al. 2004).

Heavy metals may be present in the soil naturally or can be added to soil by anthropogenic activities. Volcanic emissions and weathering of metal-enriched rocks by air can add large amounts of heavy metal to soil. In addition to this, human activities like exploitation of mines, use of metal-based pesticides and metal-laced sewage sludge in agriculture, combustion of fossil fuel, metallurgical and electronics industries and military weapons also contribute towards heavy metal contamination of soil (Oves et al. 2016).

2.1 Different Heavy Metals, Their Sources and Exposure

2.1.1 Lead (Pb)

Sources

Lead is naturally present in little concentrations in the earth's crust. Anthropogenic activities such as burning of fossil fuels, mining and manufacturing are major sources of lead. Lead-acid batteries, ammunitions, metal products (solder and pipes) and devices to shield X-rays also contain lead (Table 1). In the present day, the major source of lead poisoning in children is dust and chips from deteriorating lead paint on interior surfaces (Lanphear et al. 1998).

Exposure

Exposure occurs chiefly via inhalation of dust contaminated with lead and intake of lead-contaminated food, water and paints (ATDSR 1999). Lead is considered as the most systematic toxicant. Age and physiological status influence the lead absorption as children are more prone. The highest proportion of lead is taken into kidney followed by the liver and the other soft tissues such as heart and brain, and is present in considerable quantities in human skeleton (Flora et al. 2006).

Table 1 Summary of chemical forms, sources, mode of exposure and ill effects of specific heavy metals on human health

	Lead	Arsenic	Cadmium	Mercury	Chromium
Chemical form	Pb^{2+} Oxidation states: +2, +4	Trivalent arsenic As^{III}, pentavalent arsenic As^{V} and organic forms—methylated metaboblites, e.g. MMA, DMA and trimethyl arsenic oxide Oxidation states: +3, +5	Elemental form (Cd) Oxidation state: +2	Elemental (Hg), organic (methylmercury) and inorganic (Hg^{+}) Oxidation states: +1, +2	Divalent to hexavalent chromium Trivalent chromium Cr(III)—most stable Oxidation states: +2, +3, +4, +5, +6
Sources of heavy metal	Burning of fossil fuels, mining, industrial uses (batteries, ammunitions, devices to shield X-rays), agricultural and domestic use	Agricultural applications (insecticides, fungicides, algaecides), and industrial uses (wood preservatives, dyes, metallic ores, glassmaking)	Sedimentary rocks, marine phosphates, industrial manufacturing of alloys, pigments and batteries. Cigarette smoke Traces in leafy vegetables—potatoes, grains and seeds	Combustion of fossil fuels, industrial uses (batteries, thermometer, dental amalgams) and agricultural (fertilisers) applications	Industrial uses (metal processing, tannery, chrome production, stainless steel welding)
Mode of exposure	Inhalation and ingestion	Inhalation, ingestion, dermal contact and parenteral route (to some extent)	Inhalation and ingestion	Inhalation, ingestion and dermal contact	Inhalation, ingestion and dermal contact
Ill effects on health	Affects almost all organs (systemic toxicant), nausea, frequent vomiting, thirst, diarrhoea or constipation, anaemia (inhibits biosynthesis of haemoglobin), change in RBC morphology, miscarriage, disruption of nervous system	Tumour of lungs, skin, liver, bladder and kidneys, gastrointestinal pain, fever, damage of mucosal membrane, weakness and even death	Lifetime accumulation, osteoporosis, anaemia, emphysema, renal injury, eosinophilia, cancer of lungs, prostrate, pancreas and kidneys	Neuro, renal and gastrointestinal toxicity with gastric ulcers and haemorrhage and effects on reproductive system Vomiting, diuresis and insomnia	Irritation and ulcers of stomach and small intestine, anaemia, sperm damage, male reproductive system damage, renal and hepatic damage

MMA monomethyl arsenic acid, *DMA* dimethyl arsenic acid, *GSH* glutathione synthetase, *DNA*, *ROS* reactive oxygen species, *RNS* reactive nitrogen species

2.1.2 Arsenic (As)

Sources

The major inorganic forms of arsenic include the trivalent arsenite and the pentavalent arsenate. The organic forms are the methylated metabolites—monomethylarsonic acid (MMA), dimethylarsinic acid (DMA) and trimethylarsine oxide. The major sources are natural phenomena such as volcanic eruptions and soil erosion, and anthropogenic activities. Arsenic is used in agricultural applications such as insecticides, herbicides, fungicides, algicides, sheep dips, wood preservatives and dye stuff and in veterinary medicine for the eradication of tapeworms in sheep and cattle (Tochounwou et al. 2012; ATSDR 2000).

Exposure

One can be exposed to arsenic by ingestion, inhalation, dermal contact and parenteral route to some extent (Table 1). However, food is reported to be the largest source of arsenic poisoning, with an average intake of about 50 μg/day. Those involved in workplaces utilising or manufacturing arsenic such as vineyards, ceramics, glassmaking, refining of metallic ores, pesticide manufacturing and application, wood preservation and semiconductor manufacturing are more prone to arsenicpoisoning (NRC 2001).

2.1.3 Cadmium (Cd)

Sources

Cd is a heavy metal which is widely distributed in the earth's crust. Sedimentary rocks and marine phosphates are major sources of cadmium, containing approximately 15 mg cadmium/kg. Cadmium used in manufacturing of alloys, pigments and batteries is also a source of contamination (Galan et al. 2001).

Exposure

Inhalation of cadmium or cigarette smoke and ingestion of food contaminated with Cd are general routes of entry of cadmium in humans (Table 1). Occupation in primary metal industries and cadmium-contaminated work places makes people more prone to cadmium toxicity. Emissions from mining, smelting and manufacturing of batteries, pigments, stabilisers and alloys are some other sources of exposure (ATSDR 2008). In trace amounts, it can also be found in some eatables such as leafy vegetables, potatoes, grains and seeds (Satarug et al. 2003).

2.1.4 Mercury (Hg)

Sources

Hg commonly known as quicksilver is the only metallic element that is liquid at standard conditions for temperature and pressure. Mercury exists in three forms (elemental, inorganic and organic) and each one is toxic. The most common form in environment is methylmercury, formed by the methylation of inorganic forms of Hg by microorganisms found in soil and water. Human and animals are exposed to elemental mercury vapour (Hg^0), inorganic mercurous (Hg^{1+}), mercuric (Hg^{2+}) and organic mercury compounds. Mercury is commonly used in the electrical industry (switches, thermostats and batteries), dentistry (dental amalgams), production of caustic soda, nuclear reactors, antifungal agents for wood processing and preservative of pharmaceutical products (Table 1) (Zahir et al. 2005; Sarkar 2005).

Exposure

The main factors for the exposure of all forms of Hg are environmental pollution, contaminated food (fish consumption), dental amalgams, medical care, industrial and agricultural operations, and occupational operations. Water is contaminated by Hg due to off-gassing from the earth's crust and also through industrial pollution. Hg is methylated by algae and bacteria in water which is consumed by fish and shellfish and subsequently enters food chain. Once absorbed it has a very low excretion rate and most of it usually accumulates in the kidneys, neurons and liver (Sarkar 2005).

2.1.5 Chromium (Cr)

Sources

Cr occurs in environment in different oxidation states ranging from chromium (II) to chromium (VI) (Patlolla et al. 2009). Most stable form is the trivalent [Cr(III)] form and is present in ores as ferrochromite, while elemental chromium [Cr(0)] is not found in nature. The major sources of environmental chromium (mainly in hexavalent form [Cr(VI)]) are industries such as tannery, chrome plating, dyes and pigments, wood preservation, anticorrosive agents in cooking systems and boilers, and welding of stainless steel (Table 1) (Wang et al. 2006). Cr (VI) has also been reported in ground and surface waters and the values are exceeding the World Health Organization limit for drinking water of 50 μg of Cr (VI)/L (Velma et al. 2009).

Exposure

Occupational exposure is the matter of concern as there has been high risk of Cr-induced diseases in industrial workers who are occupationally exposed to Cr(VI). Exposure occurs orally through contaminated food and water, through dermal contact and via inhalation. The concentration of chromium in food varies and depends on processing and preparation and generally fresh foods have concentrations ranging from <10 to 1300 μg/kg (Guertin 2005).

3 Effect of Heavy Metals

3.1 Effects of Heavy Metals on Plants

Heavy metal toxicity adversely affects the plant health. The metals like Zn, Ni, Cu, V, Co, W and Cr when exceeding trace levels are highly toxic to plants (Schutzendubel and Polle 2002). Metal toxicity varies with plant species, specific metal, their concentration, chemical form, soil composition and pH (Sharma and Agarwal 2005). Heavy metals reduced the plant growth by inducing adverse impact on plant at physiological, biochemical and genetic levels. Some of the heavy metals are genotoxic to plants. Low pH of soil facilitates the accumulation of heavy metals in soil making it more genotoxic than soil with higher pH. Higher level of heavy metal in soil not only affects plant and human health but also exerts adverse effects on microbial composition of soil and soil enzyme activities (Wani et al. 2007). Metal genotoxicants can induce the chromosomal aberrations in plant cells. Pb, Cd and Hg metals were observed to induce C-karyokinesis, polyploidy, fragmentation of chromosomes, formation of micronuclei and decomposition of nuclei in *Allium cepa* and *Allium sativum* (Liu and Kottke 2004). Yi et al. (2010) reported the Al-induced chromosomal aberrations, formation of micronuclei and cell cycle dysfunction in root tips of *Vicia faba.* On exposure to heavy metals considerable decrease in the rate of synthesis of DNA, RNA and histone proteins was observed in plants (Oves et al. 2016).

At physiological and biochemical levels heavy metals retard the plant growth by disturbing different cell organelles, inducing lipid peroxidation of membranes (Meisrimler et al. 2011), deactivating photosynthesising enzymes and pigments (Srivastava et al. 2012; Bibi and Hussain 2005; Diwan et al. 2012), altering permeability of cell wall and plasma membranes (Kabala et al. 2008), etc. Nickel (Ni) in increased concentration is a potential inhibitor of photosynthesis and decreases the chlorophyll content and stomatal conductance. Excess of Ni also affects root anatomy and induces the accumulation of proline. Further Ni-induced peroxidation of membrane lipids is associated with extensive degradation of intracellular membranes and organelles, particularly chloroplasts (Lin and Kao 2007; Maksimović et al. 2007). Similarly when Zn gets accrued in plant tissues, it causes alteration in vital growth processes such as photosynthesis, chlorophyll biosynthesis and membrane integrity. An excess of Zn had been reported to have a negative effect on mineral nutrition and mineral uptake. At higher concentration, Zn inhibited root growth and Fe, Mg, K, P and Ca translocation. In addition to this Zn also induced chlorosis and necrosis of young leaves, disintegration of cell organelles and disruption of membranes and increased the number of nucleoli (Rout and Dass 2003).

Cu at toxic concentrations interferes with numerous physiological processes. It causes damage to cell membranes by binding to the sulphydryl groups of membrane proteins and by inducing lipid peroxidation (Chen and Kao 1999). In addition to this Cu also induces interveinal foliar chlorosis and inhibits root growth and production of root hairs. Toxic effects of Cu on *Withania somnifera* were echoed by the reductions in fresh weight, root and shoot length and pigment contents and altered activities of antioxidative enzymes (Khatun et al. 2008). Cr is another major metal pollutant of both soil and water, which is released mainly from the leather tanning, textile and electroplating industries. Usually Cr occurs in two forms: trivalent Cr (III) and hexavalent Cr (VI), and both forms are taken up by plants. Cr (III) is sparingly soluble and less toxic, while Cr (VI) being more soluble in water is highly toxic to biota and produces severe oxidative stress. Cr interferes with several metabolic processes, causing toxicity to the plants as exhibited by reduced root growth and biomass, chlorosis, photosynthetic impairing, stunting and finally plant death. Greater binding efficiency of Cr(III) results in the formation of stable Cr(III) complexes with proteins and nucleic acids, which consequently leads to inhibition of DNA replication and RNA transcription (Ahemad 2015).

Heavy metal toxicity is attributed to its entry within the cell, and reduction in the cell producing ROS and free radicals. The excessive production of ROS such as superoxide radical ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and alkoxyl radical ($RO^{\cdot}$) further aggravates the metal toxicity (Kandziora-Ciupa et al. 2013). In plants, ROS are continuously produced predominantly in chloroplast, mitochondria and peroxisomes. Production and removal of ROS have been balanced. However the production and scavenging of ROS might be disturbed by the number of biotic and abiotic factors including heavy metal stress (Apel and Hirt 2004). These ROS had the capacity to initiate lipid peroxidation and degrade proteins, lipids and nucleic acids. Ultimately these ROS might lead to the death of plant cell by enhancing the expression of the ROS-dependent and cell death-related genes. Other factors like MAPK-driven phosphorylation cascades, regulatory post-transcriptional modifications such as protein oxidation and nitrosylation might be involved in ROS-dependent cell death (Breusegem and Dat 2006).

When these plants are consumed by humans these heavy metals also enter the human body and are responsible for causing several problems in humans.

3.2 Effects of Heavy Metals on Human Health

At permissible limit, heavy metals are important for enzymatic activity and genetic material integrity in biological system. For instance, Se increases the antioxidant capacity of cells by increasing the activities of superoxide dismutase and glutathione reductase enzymes as part of its protection against heavy metals (Jan et al. 2015). And iron prevents uptake of toxic metals and protects body from heavy metal toxicity. It further prevents the competitive binding of heavy metals to active sites of enzymes. Similarly, Zn acts as a cofactor for superoxide dismutase (SOD), and it protects biological macromolecules from damage caused by oxidative stress. In addition, Zn as a part of zinc-binding proteins such as metallothioneins (MTs) plays a significant role in the excretion of metals such as Pb and As from the body. Supplementation of Zn also significantly reduces the effects of $HgCl_2$ (Franciscato et al. 2011).

Hg, As, Cd and Pb are able to induce toxicity even at lower levels of exposure and are considered as systemic toxicants. Evidences of mercury toxicity are well reported in literature. Oxidative stress is amongst the major mechanisms responsible for heavy metal toxicity. These metals produce reactive oxygen/nitrogen species (ROS/RNS), which in turn may cause neurotoxicity, hepatotoxicity and nephrotoxicity in humans and animals. In-depth studies have demonstrated that metals like Fe, Cu, Cd, Hg, Ni, Pb and As possess the ability to generate reactive radicals, resulting in cellular damage like depletion of enzyme activities and damage to lipid bilayer and DNA. Each of these metals has evolved a different mode of action to imbalance normal cellular functioning (Flora et al. 2008).

3.2.1 Lead (Pb)

Pb is shown to be associated with a number of physiological, biochemical and behavioural dysfunctions in laboratory animals and humans. These include central and peripheral nervous systems, haemopoietic system, cardiovascular system, kidneys, liver and male and female reproductive systems. Perinatal exposure has also been shown to be related to behavioural and neurochemical alterations in both suckling and adult rats (Moreira et al. 2001). Studies have demonstrated that Pb-exposed animals showed increased lipid peroxidation or decrease in antioxidant defence mechanism. In a study conducted on rats, rate of lipid peroxidation in brain was enhanced on exposure to Pb (Flora et al. 2007; Adegbesan and Adenuga 2007). Although exact mechanism is not fully understood, involvement of multiple mechanisms has been evidenced from previous researches (Fig. 1).

Pb toxicity mainly affects haem synthesis pathway by inhibiting the haem and haemoglobin synthesis and by changing the RBC morphology and survival (Fig. 2). A cytosolic sulphydryl enzyme, δ-aminolevulinic acid dehydratase (ALAD), involved in this pathway is the most prone to lead toxicity and is thereby inhibited by even the low blood lead levels (about 15 μg/dL) (Zhao et al. 2007). Furthermore, lead is also reported to decrease the activity of ferrochelatase involved in the last step of haem synthesis. Inhibition of ALAD halts the conversion of δ-aminolevulinic acid (ALA) into porphobilinogen, thereby decreasing haem formation which in turn stimulates ALA synthetase, the first enzyme of haem biosynthesis by negative feedback inhibition. Consequently, there is an increased accumulation of ALA and decreased formation of porphobilinogen resulting in the circulation of ALA in blood

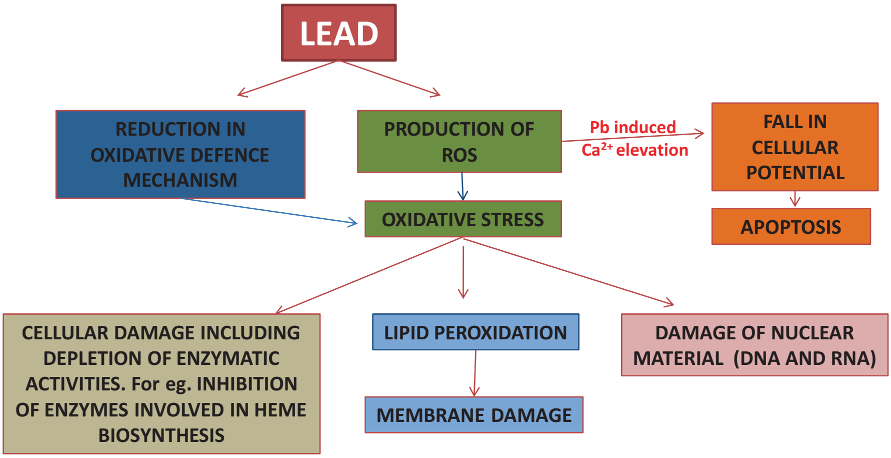


Fig. 1 Cellular effects of lead (Pb) toxicity in biological system

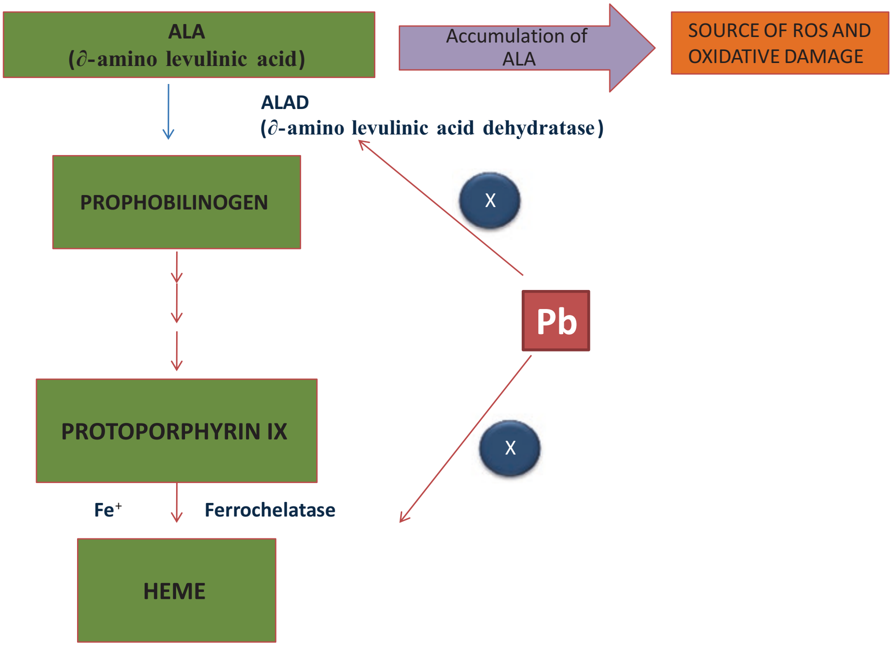


Fig. 2 Effects of lead (Pb) on haem biosynthesis pathway

and excretion in urine. It has also been reported that accumulation of ALA induces ROS generation and neurological problems such as brain damage, mental impairment and behavioural problems, neuromuscular weakness and coma (Flora et al. 2008).

3.2.2 Arsenic (As)

Arsenic toxicity is associated with a risk of developing tumours of the lung, skin, liver, bladder and kidney by enhancing the carcinogenic action of other carcinogens (Waalkes et al. 2004). In vivo studies have demonstrated the role of methylated forms of arsenic as co-carcinogens or tumour promoters (Puccetti and Ruthardt 2004). Inorganic arsenic exists mainly in two forms—arsenite (AsIII) and arsenate (AsV). Arsenite inhibits biochemical pathway by reacting with the sulphydryl groups of proteins. On the other hand, arsenate interferes with phosphorylation reactions by acting as a phosphate analogue (Valko et al. 2005). It has been reported that arsenic generates free radicals that leads to cell damage and death through the activation of oxidative sensitive signalling pathways (Shi et al. 2004). Arsenic also generates superoxide ($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), peroxyl radical (ROO), nitric oxide (NO), hydrogen peroxide (H_2O_2), dimethylarsinic peroxyl radicals $(CH_3)_2AsOO^{\bullet}$and also dimethylarsinic radical $(CH_3)_2As$. In human-hamster hybrid cells and human vascular smooth muscle cells (VSMC), generation of $O_2^{\bullet -}$ radicals has been reported on arsenic exposure. Also production of superoxide and hydrogen peroxide upon arsenic treatment in human keratinocytes cell line and vascular endothelial cells has been observed (Huang et al. 2002).

As the exact source or mechanism of ROS generation is not fully known, however, based on various hypothesis and observations, mitochondria may be considered as the major site for ROS generation. One of the supporting evidences of this is the complete disruption of the production of ROS induced by arsenite in cell lines upon addition of an inhibitor (rotenone) of mitochondrial respiratory chain. In addition, ubiquinone site is also susceptible to arsenite-induced ROS generation. Arsenite binds to dithiols of pyruvate dehydrogenase (PDH) in both the pure enzyme and tissue extract and inhibits its activity (Shi et al. 2004).

Arsenic damages membrane lipids and DNA, thereby also generating reactive nitrogen species (RNS) (Valko et al. 2005). On arsenic toxicity free radicals are reported to be generated by flavin enzymes such as NAD(P)H oxidase and NO synthase. In an experiment on cultured cells, it is observed that arsenic upregulates NAD(P)H oxidase gene expression and translocation of Rac189, thereby enhancing $O_2^{\bullet -}$ production. Furthermore, monomethylarsonous acid synthesised from arsenic binds covalently to thiol groups of endothelial NO synthase, resulting in its enzyme activity (Balakumar et al. 2008) (Fig. 3).

Arsenic is also reported to significantly alter transcriptional factors by affecting signal transduction pathways. A number of studies on cell line demonstrated the arsenic-mediated activation of MAPK signalling through the EGFR/MEK, EGFR/Ras/MEK or Src/EGFR cascade (Son et al. 2001). Apart from this, on arsenic exposure there has been decrease in the levels of antioxidants. Many reports also evidenced that on arsenic exposure glutathione (GSH) and glutathione reductase (GR) levels decrease. GSH plays an important role in maintaining cellular redox status (Mishra et al. 2008).

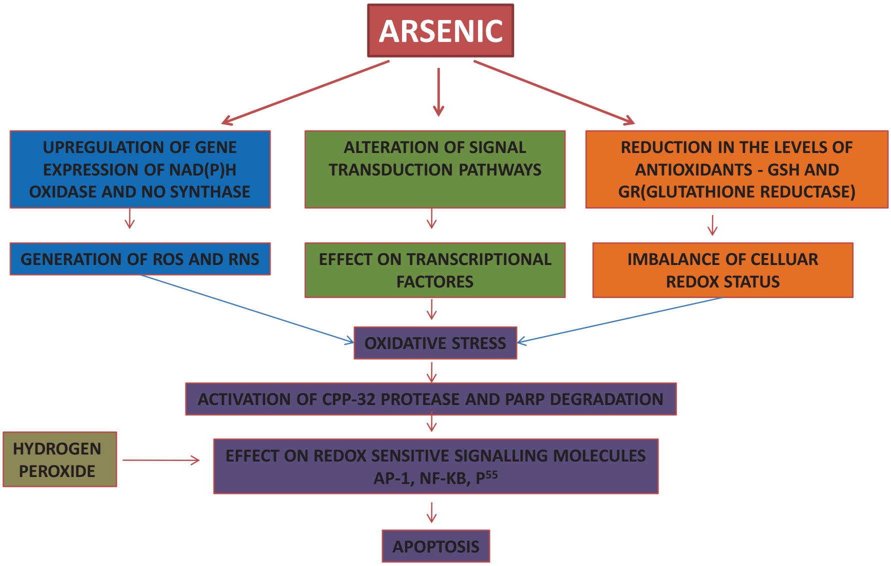


Fig. 3 Cellular effects of arsenic (As) toxicity

3.2.3 Cadmium (Cd)

Cd accumulation is toxic to kidney, liver, lungs, brain, testes, heart and central nervous system. Cadmium is considered amongst 126 priority pollutants by the US Environmental Protection Agency. The most dangerous aspect of this heavy metal is that it accumulates for lifetime as it has half-life of 17–30 years in humans (Hideaki et al. 2008). It is reported to cause osteoporosis, anaemia, non-hypertrophic emphysema, irreversible renal tubular injury, eosinophilia, anosmia and chronic rhinitis. Cd is considered as a potent human carcinogen particularly related to lung, prostate, pancreas and kidney cancers and also listed as a number one category human carcinogen by the International Agency for Research on Cancer of the USA (IARC 1993).

Cd produces superoxide radicals, hydroxyl radicals and nitric oxide radicals indirectly rather than directly as in case of other heavy metals. Reports evidence that Cd could replace iron and copper from a number of cytoplasmic and membrane proteins like ferritin, thereby releasing and increasing the concentration of unbound iron or copper ions which eventually catalyses the Fenton reactions causing oxidative stress (Watjen and Beyersmann 2004). Cd is reported to cause toxicity by binding the imidazole group of the His-74 of enzyme SOD which is crucial for degeneration of hydrogen peroxide. Cd replaces Mn(II) ions of liver mitochondrial MnSOD and thus inhibits its activity. Cd also deactivates DNA mismatch repair activity (MMR) (McMurray and Tainer 2003) which results in cellular errors and birth defects (Fig. 4).

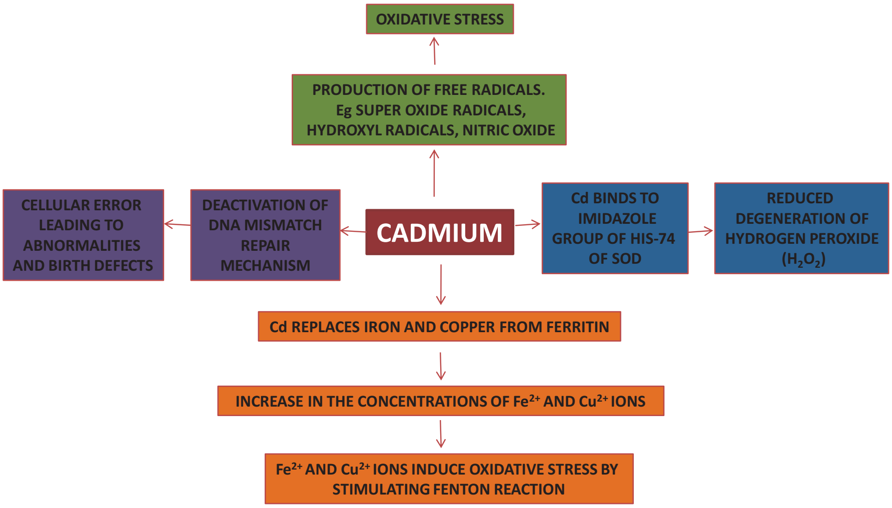


Fig. 4 Cellular mechanism of cadmium (Cd) toxicity

3.2.4 Mercury (Hg)

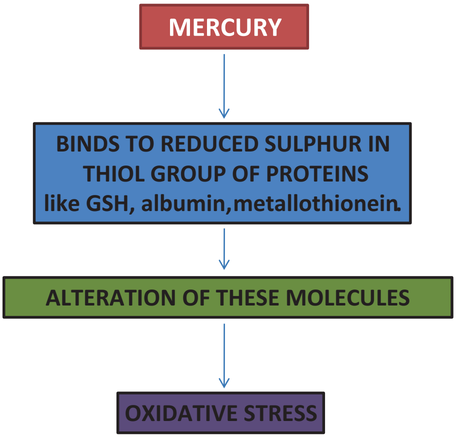
All forms of mercury (elemental, inorganic and organic) have toxic effects in a number of organs, especially in the kidneys, and are associated with neurotoxicity, nephrotoxicity and gastrointestinal toxicity with ulceration and haemorrhage. However, organic Hg is less toxic for the kidneys. Mercuric ions are reported to bind to reduced sulphur especially in the thiol groups of glutathione (GSH), cysteine, albumin and metallothionein (MT) and thus alter the structure of these molecules (McGoldrick et al. 2003) (Fig. 5). This alteration induced oxidative stress and lipid peroxidation in kidneys of rat.

On exposure of Hg, detrimental effects have also been seen in oligodendrocytes, astrocytes, and cerebral cortical and cerebellar granular neurons obtained from embryonic and neonatal rat brains. A number of stress proteins (such as heat-shock proteins (HSPs) and glucose-regulated proteins (GRPs) are also induced under Hg stress (Goering et al. 2000).

3.2.5 Chromium (Cr)

Lungs are the primary targets of Cr toxicity and it has also been reported to cause multiorgan toxicity such as renal damage, allergy and asthma, and cancer of the respiratory tract in humans. Inhalation causes irritation of the nose and can also cause nose ulcers. The major deleterious effects of Cr (VI) exposure are stomach and small intestine irritation and ulcers, stomach tumour, anaemia and damage of sperms. On the other hand, Cr (III) compounds are not much harmful. However, in

Fig. 5 Effect of mercury (Hg) on cellular proteins



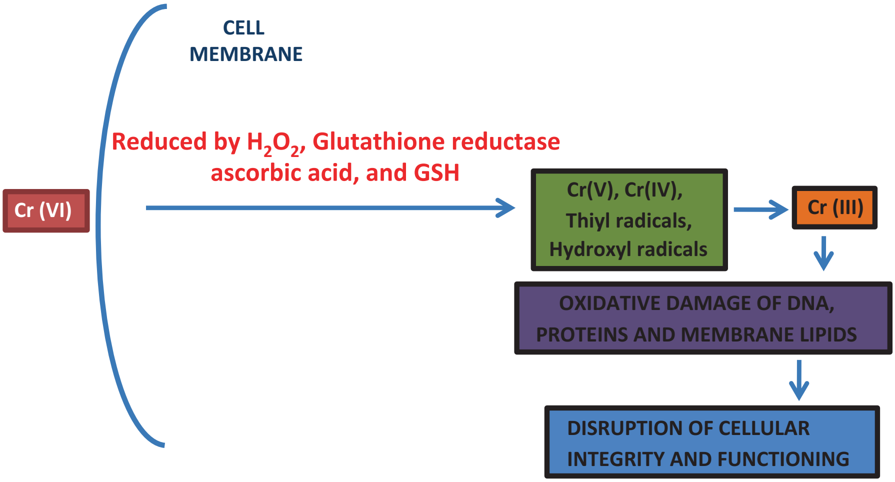


Fig. 6 Cellular effects of different form of chromium (Cr)

some sensitive persons, allergic reactions such as redness and swelling of the skin have been reported (Tochounwou et al. 2012).

It has been demonstrated that Cr(VI) can pass through cell membranes and is reduced by hydrogen peroxide (H_2O_2), glutathione (GSH) reductase, ascorbic acid and GSH to produce reactive intermediates, including Cr(V), Cr(IV), thiyl radicals, hydroxyl radicals and ultimately Cr(III). These are proved to damage DNA, proteins and membrane lipids and eventually disturb cell integrity and functioning (De-Mattia et al. 2004) (Fig. 6).

4 Different Techniques for the Treatment of Heavy Metals

The accumulation of heavy metals in the environment results in various toxicity symptoms. Various physical, chemical and biological methods have been employed to remove or reduce the heavy metals from the metal-contaminated soils/waters (Pan et al. 2018). The diverse mechanisms of metal removal have been discussed below and Fig. 7 summarises these methods.

4.1 Physical Methods and Their Drawbacks

The methods such as adsorption, mechanical screening, excavation, hydrodynamic classification, in situ fixation (or stabilisation), flotation, and magnetic or electrostatic separation which physically treat/remove the metals from contaminated soil/water are referred to as physical methods (Lambert et al. 2000; Gunatilake 2015; Sharma et al. 2016; Pan et al. 2018). The physicochemical treatment used for heavy metal removal from the metal-polluted wastewaters is called as adsorption method. The adsorption is an effective, relatively low-cost process in which the plant waste products have been chemically modified as specific adsorbents to enhance their adsorption efficiency (Sharma et al. 2016).

The industrial wastewaters polluted with heavy metals may be treated with the chemically pretreated modified adsorbents or with natural adsorbents such as activated carbons, zeolite (clinoptilolite), sepiolite, kaolin and montmorillonite clay. It has been observed that cationic heavy metals may be removed by the natural zeolites and such removal through such adsorbents has been affected by both hydrated ion diameter and charge density (Erdem et al. 2004). Besides this, the

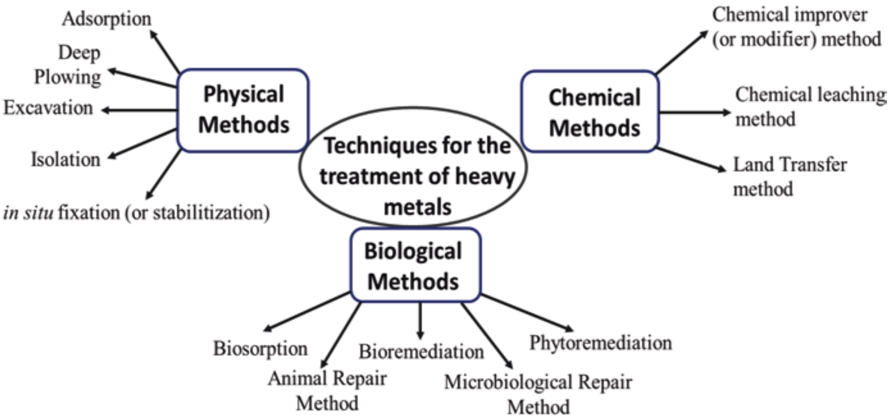


Fig. 7 Various methods of heavy metal removal from the environment

surfactant (sodium dodecyl sulphate)-modified montmorillonite may also be used for the removal of Cu^{2+} and Zn^{2+} heavy metals (Lin and Juang 2002). Divalent cationic metal ions are removed from the polluted waters due to electrostatic forces leading to adsorption or ion exchange through a natural clay (fibrous) mineral, sepiolite (Lazarević et al. 2007). The sepiolite has been suggested to be effective for metal ion removal (Pb^{2+}, Cd^{2+} and Sr^{2+}) from polluted wastewaters due to its adsorbent capacity. The untreated sepiolite was more effective in adsorbing, retaining and removing the metal ions when compared to partially acid treated sepiolite.

Jiang and co-workers (2010) reported that kaolinite clay has the potential to adsorb cationic metal ions (Cd^{2+}, Ni^{2+}, Pb^{2+}, Cu^{2+}) and can be potentially used to remove these cationic ions from the polluted wastewater. Further, it was observed that increase in pH enhances the metal ion removal and with the application of kaolinite the concentration of Pb^{2+} was dropped about 50% (from 160 to 8 mg/L). The metal-contaminated sites may also be remediated through excavation which is one of the oldest physical methods of clean-up of contaminated soils. Although this method completely removes the pollutants from the soil yet it is the most expensive and unsafe method for removal/disposal of contaminants (Lambert et al. 2000).

4.2 Chemical Methods and Their Drawbacks

In the chemical methods, the contaminated soils/water may be treated with some chemicals which act as modifiers to reduce/remove the heavy metal ions or toxicants (Lambert et al. 2000). In the on-site or in situ method, the heavy metals are treated on the contaminated site by adding chemicals which convert the toxicants to the least harmful state. This technique is generally referred to as stabilisation or in situ fixation method. For example, phosphate fertiliser may be added to the metal-contaminated soils. The phosphate reacts with lead or heavy metal ions to form insoluble mineral pyromorphite or heavy metal phosphates (Lambert et al. 1997). Due to insoluble nature, these heavy metals do not spread further into the ecosystem or food chains.

4.3 Biological Methods

The physical and chemical methods for the removal and treatment of metal ions from the polluted wastewaters or soil or environment have been observed to be non-eco-friendly and relatively expensive. Also, the implications of these methods have been limited by various factors and generally affected by the concentration of metal ions in the polluted waste. Therefore, an environmental-friendly, cost-effective, biologically potent and efficient alternative method has been desired for the removal of metal ions from the industrial effluents and polluted ecosystem (Aksu et al. 1992; Sharma et al. 2016).

The industrial effluents may be treated through precipitation, complexation, chelation, coordination, electrochemical, membrane or ion exchange methods (Abdi and Kazemi 2015; Chen 2013; Sharma et al. 2016; Thakur and Semil 2013). Besides these technologies, the biosorption technique has also been used to remove the heavy metals from wastewaters. In this biological method, an eco-friendly, efficient, low-cost, dead/inactive biosorbent preferably which does not produce any secondary metabolite is used for the passive uptake of toxic metal ions. The dissolved metal species are being sorbed by biosorbent (solid phase) and solvent (generally water, liquid phase). Principally, the removal of metal species is dependent on the attraction of sorbent by the sorbate (Sharma et al. 2016). However, the success of this technique is also dependent on various factors like type of biological material used (living or dead), pH, biomass concentration, initial heavy metal concentration and temperature (beyond range 20–35 °C) (Aksu et al. 1992). The slight variation in pH may change the functional groups' activity, thereby affecting the solutions' chemistry and competitiveness for the heavy metal ions (Galun et al. 1987). It has been reported that biosorbents are relatively most cost-effective and highly efficient alternative method when compared to other biological materials (algae, bacteria, fungi and yeast) for heavy metal removal from aqueous solutions (Sharma et al. 2016).

The method that employs biological materials such as algae, bacteria, fungi, yeast and plants for metal removal or clean-up strategies is referred to as bioremediation (Vidali 2001). This is the most effective harmless alternative of various chemical and physical methods. Bioremediation is a technique which needs least capital investment and relatively low technology, widely accepted and directly carried out at the contaminated area. In this technique, environmental contaminants and/or organic wastes are subjected to biological degradation (enzymatically) to innocuous levels with the help of living microorganisms and plants. When the microbes are subjected to contaminated lands/areas for on-site degradation of contaminants then the process is referred to as bioaugmentation. Bioremediation techniques may be categorised as in situ or ex situ depending upon the site of application. Those techniques which are applied to on-site soil/groundwater are called as in situ method whereas ex situ is that technique which is applied when soil/groundwater is imported through either excavation or pumping (Vidali 2001).

Brown algae have been proven to be the most efficient and potential alga for the uptake of heavy metals due to its high substrate specificity, appropriate mechanical properties, storage polysaccharides, specific biochemical and cell wall compositions. The cell wall of brown algae constitutes amino-carboxyl-sulphonate and sulphydryl groups that are involved actively in metal removal (Davis et al. 2003; Sharma et al. 2016). Also, fucoidan and alginate in its cell wall promote the chelation and sequestration of heavy metal ions. Further studies have emphasised that consideration of pretreatment, factors affecting biosorption capacity, immobilisation and introduction of specific molecular tools for development of engineered algal strains with higher selectivity and biosorption capacity and development might develop cost-effective, highly efficient micro- and macroalgae (Zeraatkar et al. 2016). Besides brown algae, the biosorption potential of bacteria and yeast is also due to specific cell wall compositions. These microbes may be employed to clean effluents from various industries like mining, electroplating and textile bath. The plant secondary metabolites such as flavonoids, phytic acid, citrus pectin or alginates are also used for enhancing the process of chelation of metal ions during biological clean-up strategies (Sharma et al. 2016).

Plants have the potential to reduce heavy metals from the soil through uptake. The process of use of plants as a potential clean-up candidate is referred to as phytoremediation (EPA 1998). These processes have been preferred by the environmentalists/ecologists/scientists due to it being an eco-friendly approach and relatively more cost effective (Schnoor 1997). As compared to the method of excavation (or in situ fixation methods), the cost of phytoremediation is less than 3/4th. However, this method is a lengthy process as compared to chemical or physical processes. The phytoremediation is a very flexible technique in which plants may be employed in different ways. Like in phytostabilisation, the plants are regrown in the contaminated areas to reduce soil/wind/water erosion which spreads heavy metal contaminants (Green et al. 1997).

Some plants have been reported to be metal accumulator (also referred as hyperaccumulators) that can take up heavy metal ions through their roots and then get them accumulated in their various parts/tissues/organs due to their phytoextraction capacity (Sharma et al. 2016). Then, these plants can be harvested and safely disposed of. To further stimulate phytoextraction and phyto-sequestration, soil amendments may be added to the contaminated soils. In case of contaminated water bodies or water-rich soils/sand, aquatic plants may be directly grown to reduce/remove heavy metals and this process is referred to as rhizofiltration. Several organic acids, carboxylates, phytosiderophores, etc. have been released by the plant roots into the soils which helps in metal ion dissolution and sequestration. The metal ion mobility is hindered *via* adsorption, precipitation and accumulation through the process of phytostabilisation. Another process phytostimulation (or rhizodegradation) involves the use of plants to degrade the organic contaminants in rhizosphere with the help of enhanced microbial activity which may be induced by various root secretions. The volatile contaminants like carbon tetrachloride and ethylene dibromide may be removed with the help of plants from air, soil or water through the process of phytovolatilisation.

5 Bioremediation and Mechanism Adopted by Microbes for Heavy Metal Treatment

The term bioremediation refers to the inherent use of biological forms such as bacteria, AM fungi or yeast for treatment of polluted soils in order to detoxify them in the contaminated environment into less or non-toxic forms (Farhadian et al. 2008). Microorganisms have gained much more consciousness in recent past for actively cleaning up the contaminated environment in an eco-friendly manner (Radhika et al. 2006; Khanna et al. 2019). Bioremediation is an in situ technique that relies on the use of indigenous microorganisms for cleaning up contaminated sites via exploiting the desired activities of microbes by adjusting their growth characteristics (Bai et al. 2008). The symbiotic association of plant growth-promoting bacteria (PGPB) with plants plays an essential role in the effective bioremediation of land as well as aquatic ecosystem (Glick 2010). Plants provide a constructive environment to microbial communities in rhizosphere for remediation of different contaminants inhabiting the soil (Doran 2009). Moreover, the bacterial communities in return favours the growth of plants in different ways, thus providing them resistance against different types of environmental adversities (Prasad et al. 2010).

PGPB are scavengers of different pollutants and have the ability to lower the metal toxicities in soil and their further translocation towards shoots (Saha et al. 2017). They are also responsible for nutrient acquisition in soil-plant medium, also affecting the movement or availabilities of nutrients in soil-soil system and root-shoot system (Dotaniya et al. 2016). Moreover, they stimulate the levels of hormones in plants during metal-stressed conditions (Dontaniya et al. 2014). The metal ions have the tendency to bind the microbial cell walls through ligand interaction (Bruins et al. 2000). Some of the microbes are affected by higher concentration of heavy metals as they tend to hinder the functioning of enzymes and cause DNA and membrane damage in microbial cells (Bruins et al. 2000). On the contrary, there are several microbes that show resistance in the presence of higher levels of heavy metals. The possible mechanism of heavy metal tolerance/resistance in microorganisms is most likely due to factors such as presence of permeability barrier, sequestration, enzymatic reduction, efflux systems and chelation (Rajkumar et al. 2012). All these mechanisms contribute towards effective functioning of microbes in contaminated environment (Dotaniya et al. 2018). Out of these, the efflux system mechanism triggered through active pumps is a highly recognisable and well-known method to overcome metal toxicity (Dotaniya et al. 2018). In addition, the presence of chromosomes and extracellular DNA structure such as plasmids has also been known to show resistance towards different metal ions. The increase in the amount of organic residues in soils enhances the microbial proliferation that further improves the nutrient bioavailabilities in soil (Rajendiran et al. 2012). The solubilisation and mineralisation of the organic residues further generate C-substrates that causes biosorption of different heavy metal ions from the soil (Rajendiran et al. 2016). However, the presence of polysaccharides, glucans, chitin and proteins on cell surfaces also tends to bind different metal ions in the non-specific manner through either active or passive process and these further act as biosorbents (Rajkumar et al. 2010). The most important mechanism in bacterial membrane involves bioaccumulation process which is mediated through a number of carrier or transport proteins, ion pumps, channel proteins, complex formation and endocytosis (Dotaniya et al. 2018). The microbe-mediated metal detoxification of many metal ions also occurs through chemiosmotic action followed by reduction in the intracellular metal accumulation via active export mechanism through metal efflux systems (Dotaniya et al. 2018). Furthermore, microbial transformation of different metal ions through methylation, demethylation, oxidation and reduction is one of the important resistance mechanisms found in microorganisms (Santini et al. 2000). An outline of microbial assisted bioremediation of heavy metal ions from contaminated sites has been shown in Fig. 8.

As already discussed, plants have been found to coexist with microbes with symbiotic association where root exudates primarily play an important role in normal functioning of rhizospheric region and further influence the metal bioavailability in rhizospheric soil. The microorganisms enhance root exudation from plant roots that are C-rich and act as energy source for microbes (Dotaniya et al. 2018). The plant-microbe associations have also been employed for phytoremediation potential via protons released causing changes in pH of soils followed by the formation of organometal complexes. Moreover, it is also favoured by binding components of the cells such as amino acids, phytochelatins and organic acids (Sessitsch et al. 2013). They also alter the redox homeostasis of the rhizospheric soils by enzyme-regulated electron transport through stimulating microbial colonisation in rhizosphere (Sessitsch et al. 2013). For instance, it has been revealed that translocation and accumulation of metal ions have been favoured by citric and oxalic acid in *Echinochloa crus* suggesting their role as chelators for effective phytoextraction (Kim et al. 2010). The PGPB reduces phytotoxicities by triggering defence mechanisms, generating many growth-promoting substances such as enzymes and

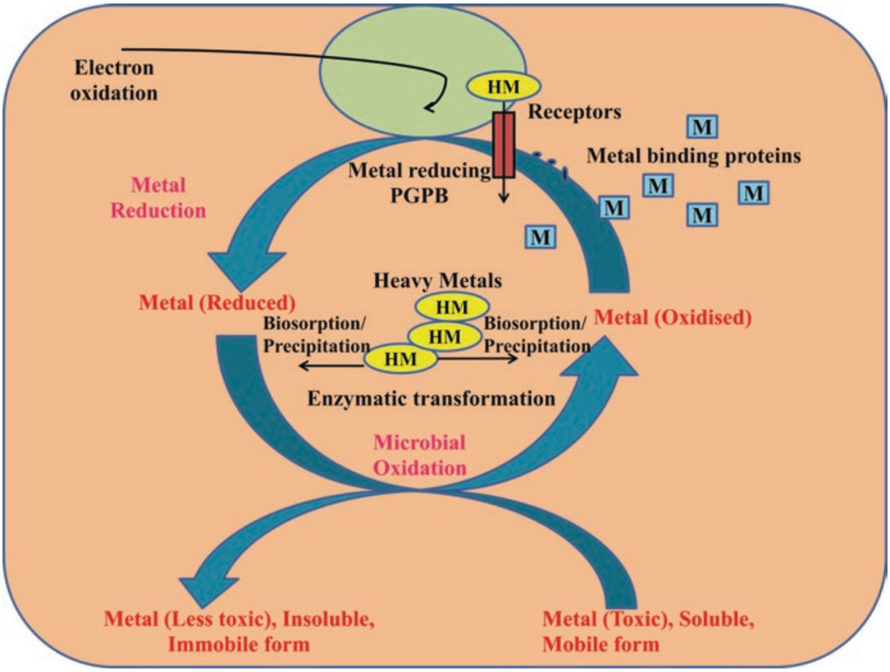


Fig. 8 Microbial mediated bioremediation of heavy metal ions

mineralising nutrients (Ma et al. 2013). They induce phytoremediation potential in many plants through improving plant biomass and mediating metal availability and its facilitation for bioaccumulation for soil-to-root and root-to-shoot translocation (Ma et al. 2016). For effective communication, PGPB generates various signals for their association with the plants in order to link up with them for creating their own environment (Hartmann et al. 2009). It mainly depends on the amount of exudates secreted in the rhizosphere for the establishment of symbiosis through chemotaxis or colonisation (Bulgarelli et al. 2013).

In addition to chemotaxis, colonisation is also favoured by generation of electrical gradients (also known as electrotaxis) across plant roots (Lugtenberg and Kamilova 2009). Recent studies have also shown that PGPB is induced in plants to obtain required amount of minerals such as Ca, Mg, Fe, K, N, P, K, Fe and B in contaminated soils, thereby establishing well-developed root system for improving phytoremediation efficiency of metal-polluted soils (Ahemad and Kibret 2014). Besides, many nitrogen-fixing bacteria such as *Rhizobium* residing in the rhizosphere also improve the fertility of soils along with the better plant growth and stimulating nitrogen concentration in plant organs (Wani et al. 2007). Similarly, AM fungi have also been reported to absorb and solubilise minerals for plant growth in Zn- and Pb-contaminated leguminous plants (Harris and Lottermoser 2006). PGPB also have the ability to transform highly toxic and insoluble forms of sulphides to available forms, thus leading to removal of heavy metals from soils through hyperaccumulator plants (Sharma et al. 2000). The secretion of siderophores by microbes during Fe-deficient conditions is yet another mechanism of promotion of Fe acquisition in different plant species (Gaonkar and Bhosle 2013). During stressed conditions, PGPB also regulate the hormone levels (IAA, gibberellins, cytokinins, ABA, etc.) in different plants that have constructive effects on plant growth and metabolism (Ullah et al. 2015). An outline of plant-microbe associations for heavy metal detoxification in the rhizosphere has been presented in Fig. 9.

Along with all these aspects, soil microflora also activate the synthesis of ethylene inhibitors (ACC deaminase), enzymes, and soluble and complex sugars to improve the plant growth under stressed conditions (Glick 2014; Naseem and Bano 2014). These metabolites play an essential role in plants to overcome stressed conditions. Amongst all, plant improvement is highly favoured through inoculating different microbes under stressed conditions. Further, various advanced biotechnological tools and applications should be envisaged to exploit their role for stress resistance and plant growth and development.

6 Different Methods Used to Increase the Microbial Efficiency for Heavy Metals

Heavy metals are not completely degraded; rather they are transformed from higher oxidation state to inorganic complexes. The most appropriate method by which this transition can be achieved is mediated through microbial remediation (Emenike

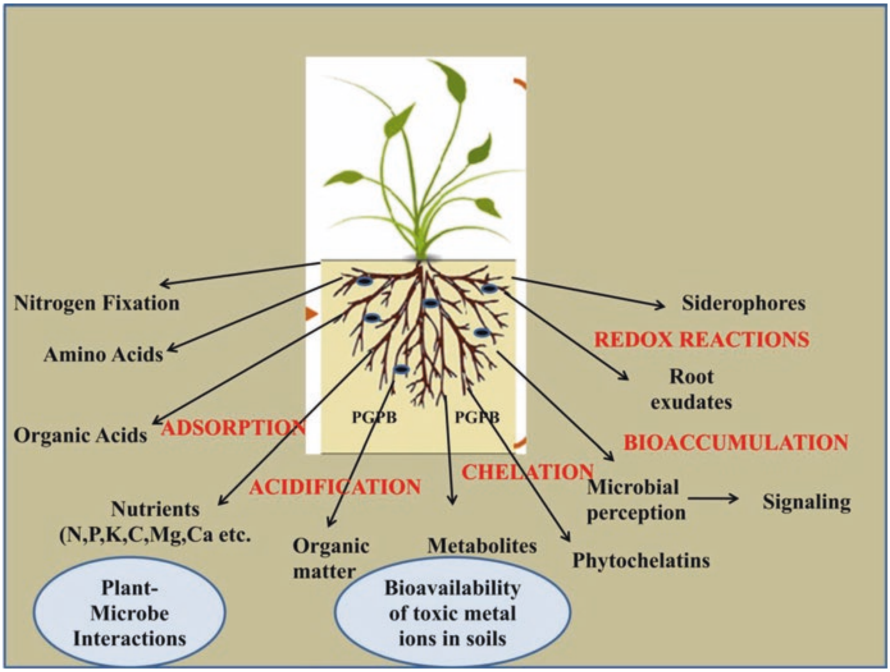


Fig. 9 Outline of plant-microbe associations for heavy metal detoxification in the rhizosphere

et al. 2018). Taking microbial remediation into consideration, it has been known to be the most potent and eco-friendly method for remediating soils polluted with different pollutants. According to the positive effect of microbes on living forms, bioremediation is one of the most promising and sustainable remediation technologies for removal of xenobiotics and heavy metal contaminants from the soil that replenishes and rectifies the soil nutrients (Dadrasnia et al. 2015). The defence response of microbes against heavy metals is a complex process that depends on the concentration and availability of metal ions and various factors such as type of metal, medium, microbial species and facilitation type (active or passive) (Tak et al. 2013). Various other factors such as polysaccharides, proteins, lipids, receptors and functional groups (carboxyl, amido, amino, hydroxyl, etc.) are also involved during binding action of different metal ions onto microbial surfaces (Roychowdhury et al. 2019). This process of bioremediation can be carried out in situ or ex situ in soil, wastewaters, sludge and sediments. In ex situ bioremediation, the contaminants are removed physically at the site followed by the in situ treatment, comprising a number of tools and technologies for the final treatment. There are many factors that influence this process: (a) microbial inoculum, (b) loss of viability of microbe used, (c) cell death after inoculation, (d) antagonistic relationship with other microbes and (e) pH, temperature and humidity (Liu et al. 2009; Thompson et al. 2005; Emenike et al. 2018). The microbes used during bioremediation transform the metal ions from one oxidation state to water-soluble, less toxic form; thereby, the metal ions precipitate and become less available followed by their removal from the contaminated site (Knox et al. 2000). Several microbes involved in the bioremediation process include *Pseudomonas, Bacillus, Azotobacter, Rhizobium, Streptomyces, Aspergillus, Saccharomyces, Streptoverticillium*, etc. (Nanda and Abraham 2011; Tunali et al. 2006). Removal of heavy metals by microbial association can be formulated through several methods such as bioaugmentation, biostimulation and bioattenuation as discussed below:

6.1 Bioaugmentation

Bioaugmentation refers to improvement in the degradation abilities of polluted sites through inoculation of potent microbes or microbial consortium. The prerequisite of this technique is addition of proper microbial inoculum, addition of genetically engineered PGPB and incorporation of genes coding for biodegradation into a suitable vector in conjugation into microbe (El Fantroussi and Agathos 2005). This process is also affected by different biotic and abiotic vectors (Mrozik and Piotrowska-Seget 2010). It is a clean approach that involves the supplementation of indigenous PGPB onto contaminated sites for accelerating the removal or breakdown of the undesirable products. It was reported in previous studies that bioaugmentation is mainly applied into the soils with lower levels of pollutants to be degraded by the microbes. In order to degrade large quantities of contaminants an all-purpose technology of remediation is required that constitutes multistep remediation processes, including the strategies that could be detrimental for small-scale bioaugmentation process and their cost also exceeds that of normal processes (Tyagi et al. 2011). In addition, at many polluted soils, the inoculating agents, that is, microbes, are exposed to some synthetic agents such as additives for stimulating their activities (Chaperon and Sauve 2008). This could further act synergistically or antagonistically to regulate the microbial activities in soils to remediate different heavy metals. Various studies suggested that bioaugmentation technique significantly removed/reduced the different metal ion concentrations in the contaminated soils (Uche 2013).

6.2 Biostimulation

Biostimulation refers to the process encompassing the modification of the particular area of the environment to modulate the microbial activities of existing microbes within this area (Emenike et al. 2018). Therefore, biostimulation enhances the decontamination process at the particular site through the addition of one or more rate-limiting nutrient into the medium in order to trigger the degradation efficiency of inhabiting microbes at polluted site (Nikolopoulou and Kalogerakis 2018). The rate-limiting nutrients that can be added into the medium include phosphorous, carbon, nitrogen, oxygen, etc. that particularly aims at increasing the microbial proliferation suitable for bioremediation (Al Sulaimani et al. 2010). One of the advantageous factors behind this process is that the native population of microbes at the site induce the detoxification process because of the adaptability to the surface and subsurface environment (Atagana 2008). Other environmental factors such as optimum levels of pH, humidity, temperature, oxygen, pollutant type and soil properties also affect the activity of biostimulation (Atlas 1981; Bundy et al. 2002). A study reported by Fulekar et al. (2012) conducted biostimulation in heavy metal-contaminated soils. They isolated the metal-resistant bacteria from the sites and inoculated into the bioreactor comprising different heavy metals (Cd, Cu, Fe, etc.) with different concentrations and anaerobic conditions that were maintained for the experimentation. They found that inoculating microbial consortium was very effective in the removal of higher concentration of Cd, Cu and Fe, respectively (Fulekar et al. 2012). Another study reported by Kanmani et al. (2012) also carried out biostimulation to remove Cd through heterogeneous population of PGPB at contaminated sites. According to their study, the isolated PGPB showed plasmid-mediated Cr resistance and this process was regulated through a number of enzymes (Kanmani et al. 2012). There is a possibility to use high-performance bacteria during extreme conditions along with the genetic engineering technologies.

6.3 Bioattenuation

Bioattenuation is defined as the in situ technology for microbial remediation of heavy metal ions that utilises the ongoing natural processes for decontaminating the pollutants from chemical spills followed by reducing their levels at polluted sites (Emenike et al. 2018). Subsequently, the pollutants remain undisturbed at the site, for acquiring opportunity for its natural breakdown, reduction or conversion into the less toxic forms (Tyagi et al. 2011; Roychowdhury et al. 2019). The natural attenuation is closely related to site clean-up which also removes the sources of contaminants along with the contaminant itself. The effectiveness of this process mainly relies on the activities at pollutant sites (Dogra et al. 2018). Nevertheless, the variation in this process also occurs, depending on the physical, chemical and biological properties of the soil and water systems. It can reduce the heavy metal pollution through biodegradation and chemical transformation, ultimately removing the pollutants via dispersion method or simple dilution method. Moreover, it involves binding of contaminants (metal ions) onto soil particles (adsorption) in order to prevent the migration of metal ions into distant parts. It is the most effective, inexpensive and appropriate method for removal of metal contamination from different sites based on natural processes in order to dissipate pollutants via biological transformation.

7 Different Plant-Microbe Interactions for Treatment of Heavy Metals

Heavy metal pollution is one of the most detrimental environmental stresses. Heavy metal stress increases the production of reactive oxygen species leading to generation of oxidative stress in plants. It also leads to lipid peroxidation, dysfunctioning of mitochondria, disruption of membrane integrity and decreased photosynthetic efficiency (Akhtar et al. 2018a, b). Plants have well-developed defence mechanism to act against the increased ROS and metal toxicity which includes metal chelation, its sequestration and alteration in antioxidative defence mechanisms (Jalmi et al. 2018). In the rhizosphere, ample microbial population is present which has great potential in mitigating and managing plant survival in normal as well as stressed conditions.

Plant growth-promoting rhizobacteria (PGPR) play a very vital role in plant growth, remediating heavy metal-contaminated soil (Raj Kumar et al. 2012). PGPR enhances the mobility of heavy metals, acidifies the rhizospheric zone and alters root surface area to increase heavy metal availability. Ma et al. (2016) in their study reported the significant role of PGPR in mitigating heavy metal toxicity. These PGPR showed a large number of responses to heavy metals like biosorption of metal, its bioaccumulation, reduction or oxidation and transformation. Various plant microbes interact in the rhizosphere and play a role in reducing the heavy metal toxicity (Table 2).

8 Role of Genetically Modified Microorganisms in Bioremediation

With the change in climatic conditions, there is a promising need to remediate the contaminated environment and to protect crops from the contaminants. Reduced degradability of microbes and accumulation ability of plants limit the process of bioremediation. Genetical engineering of living organism is one of the efficient and highly decisive perspectives of this era. Genetically modified organisms are those organisms whose gene pool has been altered in order to increase their efficiency for remediation of heavy metal toxicity. These GMOs can withstand the toxic heavy metal pollution and can be used in the process of bioremediation. Pollutant biodegrading genes are incorporated in the plants and rhizobacteria and are called as genetically modified plants and genetically engineered rhizobacteria species, respectively (Hare et al. 2017). Hirschi et al. (2000) introduced a gene from *Arabidopsis thaliana*, i.e. CAX-2 (calcium vacuolar transporter), to tobacco which increased the Ca, Mn and Cd accumulation in it. Transgenic *Arabidopsis* plants were developed by transferring *ars* C and g-ECS genes from *Escherichia coli* which enhanced the potential of arsenate uptake, reduced it to arsenite and sequestered them by making complexes with thiol peptides (Dhankher et al. 2002).

Table 2 Plant-microbe interactions under heavy metal stress

Heavy metals	Plant	Microbe	Effect of plant-microbe interactions	References
Cr	*Lens culinaris*	*Bacillus anthracis, Bacillus cereus*	Enhanced root and shoot length, dry weight, photosynthetic pigments, number of leaves	Fatima and Ahmed (2018)
	Taraxacum platypecidum Diels, Cynodon dactylon [Linn.] *Pers*	*Rhizophagus irregularis*	Increased dry weight of the plants by 232.54% and 85.83%, respectively	Wu et al. (2014)
	Pisum sativum	*Microbacterium* sp. SUCR140	Decreased plant toxicity by reducing its bioavailability in soil, also increases plant-rhizobium symbiosis	Soni et al. (2014)
	Zea mays	*Streptomyces sp.* MC1	Enhanced dry weight by 57% and decreased Cr bioavailability by 46% and 96%	Polti et al. (2011)
	Bacillus species PSB10	*Cicer arietinum* L.	Reduced metal uptake was observed in plant resulting in improved plant growth, pigments, biomass	Wani and Khan (2010)
Cd	*Lycopersicon esculentum*	*Pseudomonas aeruginosa* and *Burkholderia gladioli*	Increased expression of various antioxidative defence enzyme genes like *SOD, POD* and *PPO* genes and decrease in expression of *CAT, GR, GST, GPOX* and *APOX* genes	Khanna et al. (2019)
	Cicer arietinum	*Bacillus subtilis, B. thuringiensis* and *B. megaterium*	Greater accumulation of metal was observed in shoot	Khan and Bano (2018)
	Hibiscus cannabinus	*Enterobacter sp. strain* EG16	Improved plant growth by decreased Cd accumulation	Chen et al. (2017)
	Solanum lycopersicum	*Funneliformis mosseae, Rhizophagus intraradices* and *Claroideoglomus etunicatum*	Antioxidative enzyme activity increased and reduction in MDA content was observed	Hashem et al. (2016)
	Glycine max	*Bradyrhizobium sp.*	Increased plant growth and reduced metal accumulation	Guo and Chi (2014)
	Zea mays	*Ralstonia eutropha*	Reduced metal uptake by immobilising it	Moreira et al. (2014)
	Sedum plumbizincicola	*Phyllobacterium myrsinacearum* RC6b	Enhanced plant growth of *S. plumbizincicola was observed*	Ma et al. (2013)
	Lycopersicon esculentum	*Magnaporthe oryzae* and *Burkholderia* sp.	Decreased metal accumulation in plants was observed	Madhaiyan et al. (2007)

(continued)

Table 2 (continued)

Heavy metals	Plant	Microbe	Effect of plant-microbe interactions	References
Ni	*Consolida ambigua* and *Calendula officinalis* L.	*Kocuria rhizophila*	Significant increase in plant growth, biomass production, chlorophyll contents and Ni heavy metal uptake	Anum et al. (2019)
	Bacillus sp. CIK-516 and *Stenotrophomonas* sp. CIK-517Y	*Raphanus sativus*	Improved plant growth, dry biomass, chlorophyll and nitrogen contents, Ni uptake and also facilitates its accumulation in radish	Akhtar et al. (2018a, b)
	Paenarthrobacter sp. strain LA44 and *Stenotrophomonas* sp. strain MA98	*Odontarrhena inflata* and *O. serpyllifolia*	Bacterial inoculants with manure improved the plant growth and health, and also increased Ni uptake to facilitate the process of phytoextraction	Ghasemi et al. (2018)
	Pseudomonas putida	*Eruca sativa*	Increased root and shoot length by 34% and 41% and fresh and dry weight by 38% and 24% when compared to non-inoculated plants. Similarly, Ni uptake also showed increase by up to 46%	Kamran et al. (2016)
	Pseudomonas	*Cicer arietinum*	Reduced metal uptake by 50% when compared with uninoculated plants. Improved plant growth was observed in the plant	Tank and Saraf (2009)
	Vigna unguiculata L.	*Streptomyces acidiscabies* E13	Inhibited metal uptake, solubilisation and supply of iron to plants and protects plant from heavy metal toxicity	Dimkpa et al. (2008)
	Lycopersicon esculentum	*Magnaporthe oryzae* and *Burkholderia* sp.	Decreased Ni accumulation in plants and increased plant growth were observed	Madhaiyan et al. (2007)
	Alyssum murale	*Sphingomonas macrogoltabidus, Microbacterium liquefaciens* and *Microbacterium arabinogalactanolyticum*	Increased metal uptake into the shoot by 17% *S. macrogoltabidus,* 24% *M. liquefaciens*, and 32.4% *M. arabinogalactanolyticum*	Abou-Shanab et al. (2003)

(continued)

Table 2 (continued)

Heavy metals	Plant	Microbe	Effect of plant-microbe interactions	References
Pb	*Arabis alpina, Dysphania ambrosioides*	*Microbacterium* sp. (BXGe71)	Increased plant growth under heavy metal stress was reported	Sun et al. (2019)
	Bacillus subtilis, Bacillus thuringiensis and *Bacillus megaterium*	*Cicer arietinum*	Enhanced accumulation of metals in plant shoot. PGPR also induced Pb accumulation in plants	Khan and Bano (2018)
	R. leguminosarum, B. simplex, Variovorax sp., Luteibacter sp., P. fluorescens	*Lathyrus sativus*	Proline biosynthesis, total soluble sugars, altered activities of SOD, GPOX, CAT and APX enzymes were upregulated	Abdelkrim et al. (2018)
	Pseudomonas koreensis	*Miscanthus sinensis*	Enhanced plant biomass, photosynthetic pigments and protein content, increase in antioxidative defence enzymes	Babu et al. (2015)
	Bacillus thuringiensis	*Alnus firma*	Increased biomass, photosynthetic pigments, accumulation of heavy metal	Babu et al. (2013)

9 Conclusion: Future Perspective

As heavy metal pollution in soils is increasing at a great pace due to different human activities, there is a need for the control of these pollutants in the soil. Use of hyperaccumulating plants for the remediation of these metals is an eco-friendly and cost-effective method. Use of various microbes helps in enhancing the process of phytoremediation. More studies need to be conducted in order to understand the exact mechanism followed by the microbes for the bioremediation or accumulation of these heavy metals in plants. Use of genetically modified organisms (GMOs) is also an emerging tool where organisms are genetically modified to increase the efficiency of phytoremediation. So, there is a need for more studies to be done on how these GMOs enhance the bioremediation and the ways where they can enhance the process of phytoremediation.

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Role of Plant Growth Regulators (PGRs) in Mitigation of Heavy Metal Phytotoxicity in Plants

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Abbreviations

ABC family	ATP-binding cassette
AP2	Activator protein 2
ARF	Auxin response factors
bZIP	Basic region leucine ZIPper
CDF	Cation diffusion facilitator
CDPK	Calcium-dependent protein kinase
CYP79B3	Cytochrome P450 79B3
DREB	Dehydration-responsive element-binding protein
ERF	Ethylene-responsive factor
IRT1	Iron-regulated transporter
MYB	Myeloblastosis
NRAMP	Natural resistance-associated macrophage protein
NtCBP4	*Nicotiana tabacum* calmodulin-binding protein
PIN family	PIN-FORMED
WRKY	Containing a conserved WRKYGQK domain and a zinc finger-like motif
ZAT	C_2H_2-type zinc finger transcription factor

1 Introduction

Global environmental changes due to natural and anthropogenic activities are critically challenging the survival of earth's biotic components. Plants being special in the sense of their sessile habit are facing a continuous challenge to adopt and survive. One of the most common stressors acting on plants is heavy metal stress (HM). Rapid and progressively increasing supply and longevity of HM are the key problems of metallic pressure on biosphere. In traces they are important to the plant system but their excess amount leads to cellular damage. Excessive migration of HM into the plant system leads to metabolic disruption, physiological malfunctioning, low yield, and altered ecosystem diversity (Tchounwou et al. 2012).

HMs like Zn, Mn, Al, Pb, Ni, and Cd are indirectly responsible for the ROS production ($^{\cdot}O_2^{-}$, H_2O_2, OH); however Fe and Cu involve redox reactions to generate ROS. The indirect mechanism involves the activation of NADPH oxidases as well as cation displacement from active sites of biomolecules (Cuypers et al. 2010). Cytotoxic compounds like methylglyoxal are also known to produce during HM stress; these compounds act as oxidative stressors which leads to lipid peroxidation, biomolecule damage, as well as DNA breakdown (Barconi et al. 2011; Ahmad et al. 2012). In recent years, HMs are becoming the worldwide concern for endangering agricultural systems. Use of untreated wastewater for irrigation purposes has increased the heavy metal contamination in soil up to manifold (Lu et al. 2015). Due to these contaminations, the agricultural crops are unable to reach their full genetic potential for growth and development. Direct consumption of such HM-contaminated crops or their tissues is toxic to human as well as other animals (Damore et al. 2005).

Plants adopt different strategies to ameliorate heavy metal toxicity such as chelation, exclusion, immobilization, production of transporter proteins, stress-inducible proteins, and metal sequestration in cell organelles. Phytochelatin and metallothionein are the best known ligand-based mechanisms to uptake and accumulate both essential and nonessential heavy metals. Phytochelation is the predominant model for heavy metal detoxification as well as sequestration in plants (Anjum et al. 2015). Evidence shows that phytohormones trigger the synthesis of phytochelatins; for example brassinosteroids (BR) increase the total phytochelatin content under Pb stress in case of the algae *Chlorella vulgaris* (Bajguz 2002), and abscisic acid (ABA) regulates the synthesis of phytochelatin synthase in case of potato tuber (Stroinski et al. 2010).

Previous studies have shown that plant growth regulators like auxin, cytokinin, gibberellins, brassinosteroids, abscisic acid, jasmonic acid, and salicylic acid (PGRs) play a vital role under heavy metal stress. Exogenous treatment of phytohormones to plant under HM stress can ameliorate the toxic effects of high metal concentration (Zhu et al. 2013; Masood et al. 2016). These hormones work as chemical signals to operate complex signaling pathways that allow the plants to retain growth plasticity under biotic and abiotic stresses (Xu et al. 2016a, b). However a possible clear-cut mechanism of different signaling pathways between PGRs, heavy metals, and metal-binding ligands in plants still needs to be elaborated and explained.

2 Plant Signal Transduction and Strategies to Cope Up Heavy Metal Stress

2.1 Plant Signaling Molecules

Plants tackle varied environmental stresses which include both abiotic and biotic stresses. Out of all these stresses, heavy metal stress represents one of the most detrimental abiotic stresses. Heavy metals cause severe toxicity by targeting critical molecules and essential processes in the plant cell. One of the modes by which heavy metals cause toxicity in plants is by increased production of reactive oxygen species (ROS). To overcome such overdose of heavy metals, plants adopt multifarious strategies to boost their defense responses. These strategies include vacuolar sequestration, metal chelation, and regulation of metal uptake by various transporters, and strengthening of antioxidative defense mechanisms. Such response shown by plants is due to the intricate signaling networks executing inside the cell so as to transfer the extracellular stimulus to an intracellular response. The vital signaling components include hormone signaling, mitogen-activated protein kinases (MAPK), and calcium signaling. Besides these signaling components, other regulators such as transcription factors and microRNAs also contribute to overcoming stress caused by heavy metals (Jalmi et al. 2018a, b).

2.1.1 Calcium Signaling

Ca^{2+} acts as a secondary messenger in the normal developmental processes of the plant as well as in response to various environmental stress stimuli. Changes in free cytosolic Ca^{2+} concentration during stressful conditions trigger complex interactions and signal transduction pathways (Rudd and Franklin-Tong 2001). Numerous reports indicate alleviation of heavy metal toxicity in plants by exogenous application of Ca^{2+}. This strategy of heavy metal stress alleviation by Ca^{2+} occurs due to modulation of various physiological and biochemical processes. This includes enhanced activities of various antioxidant enzymes such as superoxide dismutase, glutathione reductase, and ascorbate peroxidase (Ahmad et al. 2015).

According to Choong et al. (2014), Cd possesses physiochemical properties very similar to Ca^{2+}. Due to high similarity in ionic radii of Ca^{2+} and Cd, there is a higher possibility of Cd uptake through receptor or voltage-gated Ca^{2+} channels. Plants exposed to Cd reveal higher level of intracellular Ca^{2+} in order to combat heavy metal toxicity (Yang et al. 2003). The strategy for heavy metal stress alleviation by increased intracellular Ca^{2+} levels occurs by production of IP_3, thereby triggering the release of sequestered Ca^{2+} from the intracellular Ca^{2+} reserves (Smith et al. 1989).

In a study on *Brassica juncea* conducted by Ahmad et al. (2015), application of Ca^{2+} was able to mitigate deleterious effects of Cd metal toxicity, thereby improving the growth, development, and seed quality. A study on *Arabidopsis* seedlings showed that Ca^{2+} attenuated toxic effects of cadmium through maintenance of auxin homeostasis indicating a cross talk between hormone signaling pathways to combat heavy metal stress (Zhao et al. 2015). In a study conducted by Arazi et al. (1999) on transgenic tobacco plants expressing the plasma membrane associated NtCBP4 exhibited enhanced levels of tolerance to Ni^{2+} toxicity. Huang et al. (2014) reported the involvement of CDPKs through transcriptional profiling of the rice roots under Cr metal stress. Increased chromium concentration was found to correlate with an increase in CDPK-like protein activity, revealing the role of Ca^{2+} signaling under stressed conditions.

2.1.2 Hormone Signaling

There are innumerable studies which report the role of phytohormones such as auxins, cytokinins, and ethylene in remodeling the root architecture under heavy metal stress. This method of root architecture remodeling can be used as an effective strategy to mitigate heavy metal stress. Wang et al. (2015) reported that under heavy metal stress plants regulated the location and accumulation of auxins by differential expression of auxin-linked genes such as CYP79B3, PIN family, YUCCA, and

ABC family. Wang et al. (2016) observed that Al metal toxicity leads to diminished root growth by inhibition of PIN2 transport from plasma membrane to endosomes leading to IAA imbalance in roots. Furthermore, *aux1-7* and *pin2* mutants showed improved tolerance to Al^{3+} in comparison to wild-type plants revealing the probable role of PIN2 and AUX1 proteins in Al^{3+} metal-induced reduction in elongation of roots.

Cytokinins (CKs) are other group of hormones which are activated under heavy metal stress to help plants mitigate heavy metal toxicity. Under Cd metal stress, reduction of photosynthetic pigments and chloroplast membrane took place. But CKs were able to restore photosynthetic capacity and primary metabolites (Piotrowska-Niczyporuk et al. 2012). Chen et al. (2014a, b, c) reported that the five most important ethylene (ET) synthesis genes in rice (OsACO1, OsACO2, OsACO5, OsACO6, and OsACS2) along with transcription factors ERF1 and AP2 were upregulated under Hg stress in *Medicago sativa*. Recently it was found that ET negatively affects Al-induced production of malate ions using ET8. This is a vital mechanism for Al tolerance (Yu et al. 2016).

2.1.3 microRNAs Under Heavy Metal Stress

microRNAs (miRNAs) are small, noncoding, single-stranded RNAs varying in size from 20 to 24 nucleotides. They play a vital role in the regulation of gene expression in a sequence-specific manner at post-transcription level (Jones-Rhoades et al. 2006). Besides playing an important role in signaling pathways during heavy metal stress, miRNAs are involved in plant growth as well. Numerous reports reveal differential expression of miRNAs under heavy metal stress (Zhou et al. 2012; He et al. 2016). It has been found that different miRNAs are differentially regulated both temporally and spatially. Recent advent of genome-wide studies, transcriptome analysis, and next-generation sequencing have been used for the identification of miRNAs which are involved in alleviation of heavy metal stress (Liu and Zhang 2012; He et al. 2016; Noman and Aqeel 2017).

In *Oryza sativa* plants exposed to Cd metal stress, differential expression of miRNAs was observed. Upon exposure to Cd, miR441 expression was upregulated whereas expressions of 12 miRNAs were observed to be downregulated. Among the down-regulated miRNAs, miR192 was found to target ABC transporter which is involved in heavy metal transport. Overexpression of miR192 decreased seed germination and plant growth under Cd metal stress in comparison to wild-type plants. This observation suggests that decrease in the levels of miR192 leads to the accumulation of ABC transcripts which ultimately causes Cd sequestration by ABC transporter (Tang et al. 2014; He et al. 2016). It has been observed that reactive oxygen species leads to lipid peroxidation under heavy metal stress. This leads to downregulation of miR397 which targets laccase. This may lead to positive regulation of lignin biosynthesis through the regulation of laccase enzyme (Jones-Rhoades and Bartel 2004).

Most of the miRNAs such as miR169, miR390, mir395, miR397, and miR528 are known to be directly involved in mitigating heavy metal stress by regulating the transcripts of ROS scavenging enzymes, laccases, or metal transporters (Xu et al. 2016a, b).

2.1.4 Mitogen-Activated Protein Kinases (MAPK)

Heavy metal stress has been found to have profound effect on MAPK signaling pathways. MAPK cascade consists of three important components, viz. MAPKKKs, MAPKKs, and MAPKs (Hamel et al. 2006). MAPKs are activated by specific metals such as Cd, Cu, and As (Rao et al. 2011; Smeets et al. 2013) and also by ROS molecules produced under such stress (Smeets et al. 2013). MAPK signaling is known to influence hormone signaling and their transport. In a report published by Singh et al. (2015), the interplay of auxin/cytokinin and MAPKs was unveiled in which OsMKK4/5-OsMPK3/6 was elucidated as a key player in auxin/cytokinin regulating the expression of OsPIN1b/9. Another study in rice showed the relationship between auxin and MAPK signaling under Cd metal toxicity. It was observed that the expression of key auxin signaling genes such as YUCCA, ARF, and PIN along with other cell cycle-related genes was negatively regulated by MAPK under Cd stress (Zhao et al. 2014).

2.1.5 Transcription Factors Under Heavy Metal Stress

Transcription factors are vital regulators of gene expression which affect various developmental processes and defense response in plants (Yanhui et al. 2006). Genome-wide expression analyses have revealed the modulation of expression of transcription factors under heavy metal stress (Farinati et al. 2010; Wang et al. 2010; Smeets et al. 2013). A number of studies reveal that MAPK signaling cascade activates the downstream transcription factors under heavy metal stress. Potential downstream targets of MAPK signaling include the following transcription factors: (a) MYB, (b) AP2, (c) WRKY, (d) DREB, (e) bZIP, (f) ZAT, and (g) ERF (Li et al. 2015).

The MYB family of transcription factors is one of the largest families in plants having diverse functions. Hu et al. (2017) reported the role of OsMYB45 transcription factor under Cd metal toxicity. They observed that mutation in *OsMYB45* leads to Cd hypersensitive phenotype of *Oryza sativa* plants. The resultant mutant showed considerable increase in H_2O_2 content in leaves with significant decrease in CAT activity in comparison to wild-type plants. In a recent study, it was observed that rice MYB transcription factor OsARM1 (ARSENITE-RESPONSIVE MYB1) regulated As-associated transporter genes. OsARM1 binds to conserved MYB-binding sites in the promoter regions of *OsLsi1*, *OsLsi2*, and *OsLsi6* which encode for essential As transporters affecting their expression (Wang et al. 2017a, b).

WRKY transcription factor binds to W-box of promoters in numerous genes responsible for abiotic and biotic stress tolerance in plants. In a study carried out by Opdenakker et al. (2012), it was found that WRKY22, WRKY25, and WRKY 29 were overexpressed under Cu metal stress. It was reported that the flagellin-induced MAPK cascade MEKK1-MKK4/MKK5-MPK3/MPK6 activates WRKY22 and its close homolog WRKY29 (Asai et al. 2002). In *T. caerulescens*, expression level of WRKY53 was found to be highly induced under Cd metal stress (Wei et al. 2008).

bZIP is another class of transcription factor which provides tolerance under heavy metal stress in plants. BjCdR15, a bZIP transcription factor from *Brassica juncea*, is a regulator of Cd uptake, transport, and accumulation in shoots and confers cadmium tolerance in *Brassica juncea* transgenic plants (Farinati et al. 2010). Recent report on a novel bZIP gene, *BnbZIP3* from *Boehmeria nivea* plant, has shown that it positively regulates heavy metal stress tolerance by overexpression resulting in improved root growth (Huang et al. 2016).

Besides the aforementioned transcription factors, Cd induces ERFs in *Arabidopsis thaliana* (Weber et al. 2006). In another report DREB transcription factors were observed, which are members of ERF family of transcription factors that get upregulated upon heavy metal exposure (Ogawa et al. 2009). Chen et al. (2016) reported that *Arabidopsis* ZAT6 acted as a positive regulator of Cd tolerance through the glutathione-dependent pathway. They revealed that ZAT6 positively regulated the expression of phytochelatin synthesis pathway genes such as GSH1, GSH2, PCS1, and PCS2.

2.1.6 Metal Transporters

Numerous metal transporters have been identified from different plants. The most important metal transporters studied are ABC transporters, NRAMP, CDF, and ZIP. ABC transporters consist of largest family. They are classified into eight subfamilies playing roles in varied cellular processes such as hormone transport, osmotic homeostasis, nutrient uptake, pathogen resistance, and metal tolerance (Park et al. 2012). *Arabidopsis* ABC transporter, AtPDR8, has been identified as Cd extrusion pump conferring resistance to Pb and Cd (Kim et al. 2007). Owing to their metal transportation capacity, ABC C-type transporters AtABCC1 and AtABCC2 have been identified as major phytochelatin-heavy metal complex transporters.

Regarding NRAMP transcription factor, in plants there are two subfamilies of NRAMP genes and several of them upregulate in Mn, Fe, and Cd deficiency. Expression analysis of NRAMP in plants suggests that unlike ZIP family (expressed mainly in roots) these metal transporters are expressed both in shoot and root, hence participating in metal homeostasis in all plant tissues (Zhang et al. 2000).

IRT1 gene from *Arabidopsis* belongs to ZIP family. It is major transporter of iron leading to high-affinity Fe uptake. In iron-limiting environment, IRT1 is present only in roots and is induced within 24 h of iron-deficient conditions. Plants overexpressing IRT1 accumulate high levels of Cd and Zn along with Fe (Connolly et al. 2002).

3 Mechanism of Action of PGRs to Alleviate HM Phytotoxicity

Plant growth regulators are a small signaling component which has an essential role at every stage of plant growth and development. Varying mechanism for different plant hormones occurs in plants. Single hormones or PGRs regulate a wide array of cellular and developmental phenomena or vice versa; that is, different hormones are involved in the modulation of single phenomenon (Gray 2004). Phytohormones crucial for plant growth and development include auxins (IAA), gibberellic acid (GA), cytokinins (CK), ethylene (ET), brassinosteroids (BRs), salicylic acid (SA), jasmonic acid (JA), and strigolactones (Bucker-Neto et al. 2017; Xu et al. 2016a, b; Colebrook et al. 2014). These plant growth regulators are universal regulators of heavy metal absorption by plants and have been used for a plethora of agronomical applications in order to alleviate heavy metal toxicity (Piotrowska-Niczyporuk et al. 2012). These hormones have imperative participation in signaling, biochemical, and detoxifying mechanism in plants exposed to heavy metal pollution (Bucker-Neto et al. 2017). They at very low concentration modulate cell wall permeability, activity of essential enzymes, growth, and reproductive behavior of plants. Higher doses of heavy metal result in lower shoot and root biomass which is by rule regulated by phytohormones (Wani et al. 2016).

3.1 Role of ABA (Abscisic Acid) in Heavy Metal Toxicity Mitigation

ABA is known as one of the most significant PGRs having an imperative role in abiotic stress management (Sytar et al. 2019a, b). It is categorized as sesquiterpene compounds and has a nonpolar configuration. The biosynthesis of ABA usually occurs during drying-up conditions and its breakdown occurs on rehydration (Roychoudhury et al. 2013). It occurs in the roots and buds of plants. The C-15 ABA basic skeleton is present in xanthotoxin, abscisic alcohol, and aldehyde, the biosynthetic precursors of ABA. A few oxidized catabolites including 8’-hydroxy-ABA, phaseic acid, and dihydrophaseic acid have a similar backbone (Roychoudhury et al. 2012). The basic physiological functions of ABA in cell-based phenomena include vegetative growth, seed germination, and responses to ecological stresses (Xiong and Zhu 2003). Moreover at cellular level they control the activity and production of various enzymes imperative to overcome dehydration (Osakabe et al. 2014), maintenance of water balance (Parent et al. 2009), and iron homeostasis (Sytar et al. 2019a, b). It is well recognized for its role in regulation of stomatal movement (Christmann et al. 2007) and hydraulic conductivity of tissues (Parent et al. 2009). It is quite evident that a plethora of developmental and physiological phenomena are notably altered by ABA. Its level is significantly enhanced in response to chilling, water deficiency, and salinity stress in various plant species (Hsu and Kao 2003).

ABA levels are observed to be augmented in response to alterations in environment conditions which further results in activation of specific signaling cascades and detoxification mechanisms (Wang et al. 2018). The content of ABA endogenously produced is elevated in plants in response to several stress signals. Furthermore, it was suggested that it might be due to upregulation of expression of genes encoding ABA synthesis from β-carotenes (Roychoudhury et al. 2012). The expression of ABA biosynthetic genes has been observed to be elevated in response to exposure to heavy metal contamination, viz. Cd, Cu, Hg, As, Pb, and Cr, which in turn elevates the endogenous content of ABA (Hollenbach et al. 1997; Sytar et al. 2019a, b). It was further suggested by Osakabe et al. 2014 that ABA transcriptomically regulates a plethora of protein-encoding genes. Various heavy metals including Al, Zn, Cd, Pb, Ni, and Cr have been reported to enhance ABA accumulation in stressed plants (Fediuc et al. 2005).

Cd, a divalent metal cation, is an utmost toxic heavy metal (Tripathi et al. 2014). The seepage of Cd ions in water, soil, and air is due to discharge from various industries, viz. mining and fertilizers, and is furthermore easily taken up by plants resulting in retardation of growth (Vishwakarma et al. 2017). Sharma et al. (2002) revealed promising results with employment of ABA-insensitive and ABA-deficient mutants to counter Cd toxicity. They further suggested that Cd stimulated growth retardation in response to elevation in ABA levels under HM stress and regulated stomatal openings. It was suggested by Hsu and Kao (2003) that the tolerance of rice plants exposed to high temperature ranging from 30 to 35 °C was enhanced in retaliation to augment ABA accumulation. Similar observations were made by Wani et al. (2016), who reported exogenous application of ABA to two *Solanum* species exotypes, viz. mining and farmland. Table 1 enlists various reports of potential role of auxins and ABA in alleviating heavy metal toxicity.

3.2 Role of Auxin in Heavy Metal Toxicity Mitigation

Plant development and its retaliation to varied environmental cues involve fine-tuning of stress amelioration strategies, which are very complex. Along with these environmental conditions faced by the plants, the interplay between heavy metal toxicity and auxin homeostasis has been widely reported. It is often indicated as an imperative regulator of several physiological processes, including growth and development (Bucker-Neto et al. 2017). It was demonstrated by Růžička et al. (2007) that auxin synthesis genes are stimulated by ethylene and trigger auxin transport to the elongation zone, modulating its response and eventually resulting in root elongation. Similar interplay between ethylene and ROS signaling was suggested by Camacho-Cristobal et al. (2015), in *Arabidopsis* plants exposed to boron deficiency stress, resulting in retardation in root cell elongation.

In plant cells endogenously auxin accelerates the degradation of AUX/IAA transcriptional repressor which bounds to the auxin-responsive factors, i.e., ARFs. It eventually results in the inhibition of transcription of AUX-ARF genes, consequently leading to alteration in plant physiology (Vanneste and Friml 2009). Auxin

Table 1 Role of auxins and ABA in heavy metal toxicity amelioration

S. no.	Plant species	HM stress	Hormone concentration	Effects	References
1.	*Vitis vinifera*	$ZnSO_4$ (0.765 μM–10 mM) or 0.22–2880 mg/L	ABA (10 μM)	– Accumulation of Zn was lowered in response to induction of ZIP gene (detoxification-related gene) – Tolerance of plant to Zn was enhanced	Song et al. (2019)
2.	*Lactuca sativa*	Cd (100 μM)	ABA (1, 5, and 20 μmol/L)	– Shoot biomass was enhanced by 0.5 μmol/L ABA treatment – Root biomass was elevated by 20 μmol/L ABA application – Lowest Cd accumulation was observed in 0.5 μmol/L ABA-applied plants	Tang et al. (2019)
3.	*Spinacia oleracea*	Cd (40 mg/Kg)	IAA (10^{-3} M)	– Enhanced fresh weight and dry mass – Elevation in Cd phytoextraction	Rizwan et al. (2017)
4.	*Trigonella foenum-graecum* L.	Cd (3 mg/Kg of soil, and 9 mg/Kg of soil)	IAA (10 and 100 μM)	– Upregulation of AsA-GSH cycle including enzymes SOD, POD, CAT, and GST – Lowered levels of ROS	Bashri and Prasad (2016)
5.	*Populus* × *Canescens*	Zn (2 μM)	ABA (10 μM)	– Root ratio of GSH-GSSG and ascorbate to dehydroascorbate was elevated – Endogenous levels of ABA, SA, and GA were enhanced – Gene expressions of *yellow stripe-like family protein 2* (YSL2) and *plant cadmium resistance protein 2* (PCR2) were increased	Shi et al. (2015)

(continued)

Table 1 (continued)

S. no.	Plant species	HM stress	Hormone concentration	Effects	References
6.	*Oryza sativa*	$NaAsO_2$ (150 μM) and Na_2SeO_4 (20 μM)	IAA (3 μM)	– Improved growth attributes – Levels of stress indicators were elevated including proteins, chlorophyll, and MDA content – Levels of cysteine and proline enhanced	Pandey and Gupta (2015)
7.	*Arabidopsis thaliana*	Cd (10 μM)	ABA (0.1–0.5 μM)	– ABA inhibits Cd uptake by roots – Expression of IRT1 in roots was significantly inhibited and hence enhanced tolerance to Cd stress	Fan et al. (2014)
8.	*Vigna radiata*	$CdCl_2$ (1, 3, 5, 7, and 9 μM)	ABA (10 μM) and IBA (10 μM)	– ABA induced enhancement in endogenous levels of IAA oxidase activity which further elevated the contents of phenols – Concentration of antioxidants, viz. CAT, POD, SOD, and APOX, was significantly enhanced	Li et al. (2014)
9.	*Arabidopsis thaliana*	Cd (50 μM)	α-Naphthalene acetic acid (NAA) (0.05 μM)	– Chlorosis symptoms were markedly reduced – Shoot growth was improved – NAA enhanced Cd^{2+} fixation and its translocation to shoots – Nitric oxide levels were repressed – Enhanced retention of Cd in roots	Zhu et al. (2013)

(continued)

Table 1 (continued)

S. no.	Plant species	HM stress	Hormone concentration	Effects	References
10.	*Triticum aestivum*	Cd (500–1000 µM)	IAA (500 µM)	– Activation of antioxidative defense system – Improved growth and pigment content	Agami and Mohamed (2013)
11.	*Oryza sativa*	Cd (0.1 mmol/L)	IAA (10^{-9}, 10^{-8}, and 10^{-7} mol/L)	– Enhanced elongation of primary and adventitious roots	Zhao et al. (2013)
12.	*Atractylodes macrocephala*	Pb (300 µmol)	ABA (2.5, 5, and 10 mg/L)	– Enhanced plant growth, levels of soluble sugars, and proteins – Activity of antioxidative enzymes, viz. SOD, CAT, POD, and APOX, was elevated – Pb uptake was lowered	Wang et al. (2012)
13.	*Raphanus sativus*	As-Zn-Cu-Co-Pb-contaminated pyrite waste	IBA (foliar spray, 10 mg/L) and (substrate application, 0.1 and 1 mg/L)	– Improved phytoremediation of metal-polluted substrates as a result of improved root and shoot growth	Vamerali et al. (2011)
14.	*Helianthus annuus*	Pb (2.5 µM) and Zn (15 µM)	IAA (10^{-12}, 10^{-11}, 10^{-10}, and 10^{-9} M)	– Root growth was elevated – Also root dry weight, root lengths, root volume, and root surface area were improved – Significant decline in metal uptake	Fässler et al. (2010)
15.	*Brassica napus*	Cd (10, 50, 100, 200, and 400 µM)	ABA (10 µM)	– ABA reduces Cd toxicity symptoms – Fresh weights of root and shoot were elevated – Lowered MDA and antioxidative enzyme levels – Internal Cd accumulation was lowered by ABA application	Meng et al. (2009)

(continued)

Table 1 (continued)

S. no.	Plant species	HM stress	Hormone concentration	Effects	References
16.	*Oryza sativa*	Pb (0.25 mM)	ABA (0.1 g/m^3)	– Restricted translocation of Pb from roots to shoots – Elevation in POD and CAT activity whereas SOD activity was lowered	Zhao et al. (2009)
17.	*Helianthus annuus*	Pb (440 mg/Kg) and Zn (128 mg/Kg)	Auxin (70 mg/L)	– Regulation of stomatal movement – Initiation of cell division and shoot growth	Tassi et al. (2008)
18.	*Helianthus annuus*	Cu (80 μM)	IAA (100 μM)	– Improved root growth in terms of root length and root hair formation – Enhanced chlorophyll and carotenoid levels – Water-use efficiency was elevated	Ouzounidou and Ilias (2005)
19.	*Oryza sativa* L.	Cd (0.5, 1, and 1.5 mM)	ABA (added directly to the culture solution)	– Cd uptake was lowered – Enhanced transpiration rate	Hsu and Kao (2003)

has a dynamic polar transport distribution and active metabolism in plant cells and aids in regulation of heavy metal toxicity. A plethora of reports suggest that heavy metal concentration in DR5-GUS receptor line revealed hormone sequestration and translocation in *Arabidopsis* seedlings in a dose-dependent manner (Wang et al. 2015) and significant participation of PINFORMED 1 (PIN 1) proteins is indicated. PIN 1 is an auxin efflux carrier for modulation of growth of root meristem or its maintenance under normal conditions (Yuan et al. 2013). Moreover comparison between Cu-treated *pin1* DR5rev::GFP mutants and DR5 REV::GFP plants revealed that the mutant showed no enhancement in auxin activity in the elongation zone and meristem, affirming the role of PIN1 in auxin distribution in metal-stress plants. Similarly, under boron stress auxin distribution in plants is altered, resulting in PIN 1 downregulation and repressed root elongation (Li et al. 2015). They further added that this alteration in root elongation might be attributed to cross talk between other phytohormones.

Another investigation by Yuan and Huang (2016) demonstrated participation of NO and auxin in the regulation of repression of root meristem growth in *Arabidopsis* plants exposed to Cd treatment. Cd induced NO sequestration, which resulted in the inhibition of auxin transport, lowering its content in root apex and consequently resulting in retardation of root meristem size. Furthermore, few reports suggest reduced auxin metabolism in response to heavy metal stress, viz. Cd-exposed *Arabidopsis* plants disturbed IAA accumulation and homeostasis, resulting in retardation in primary root elongation (Besson-Bard et al. 2009). Similar reports of disruption in homeostasis and accumulation of IAA in barley root tips were observed in response to short-term Cd exposure (Zelinova et al. 2015). Previous work also indicates that As metal treatment was able to alter the levels of three auxins, viz. IBA, NAA, and IAA, in *Brassica juncea* plants (Srivastava et al. 2013).

Exogenous application of auxin to metal-stressed plants is an imperative strategy to overcome heavy metal stress. *Helianthus annuus* plants grown in soil with moderate levels of Pb revealed enhancement in the biomass of roots and stems in response to exogenously applied IAA (Liphadzi et al. 2006). Similar observations of improvement in growth of *B. juncea* plants exposed to As stress were made by Srivastava et al. (2013). L-TRP (a precursor of auxin) when supplemented to Cd-stressed plants led to elevation in their growth attributes, i.e., plant growth and yield (Farooq et al. 2015). Effect of simultaneous application of Se and auxin on morphological and biochemical attributes of rice plants under As stress was revealed by Pandey and Gupta (2015). The combined treatment of Se and auxin was found to be more effective than individual treatment. Moreover, it was suggested by Ostrowski et al. (2016) that IAA-Asp (an auxin conjugate) affects the response of pea plants to Cd stress by alterations in CAT and POD activities, initiation of protein carbonylation, and lowered H_2O_2 concentration. Another study by Hac-Wydro et al. (2016) indicated that auxin results in modification of membrane properties, thereby alleviating toxic symptoms of heavy metal exposure. They proposed that a mixture of Pb and IAA or NAA induces decline in disruption of membrane permeability and organization and furthermore lowers heavy metal toxicity symptoms. The hemicellulose 1, a cell wall component, was found to have a key role in heavy metal stress management. Zhu et al. (2013) reported that Arabidopsis plants exposed to Cd stress and exogenous application of NAA resulted in elevation of metal retention to the roots by fixing it to the hemicelluloses. In recent literature, it was revealed that metallophytes, i.e., plants that can tolerate more metal exposure than other plants, e.g., moss *Scopelophila cataractae* requires Cu-rich environment and their survival. Under Cu-rich environment, moss reportedly accumulates more auxin and consequently activates genes required for optimal growth and cell differentiation (Nomura et al. 2014).

3.3 Role of Brassinosteroids in Heavy Metal Toxicity Mitigation

Plants inhabiting metal-polluted soils show altered metabolism, reduced growth, decreased biomass production, and metal accumulation. Heavy metals have an effect on various physiological and biochemical processes in plants (Nagajyoti et al. 2010).

Brassinosteroids play an important role in plant growth, development, and response to abiotic stress. They have the potential to protect the plants from the adverse effects of heavy metal toxicity and enhance the crop resistance in stress conditions. They improve the plant defense system by modulating the activities and contents of enzymatic and nonenzymatic antioxidants (Bücker-Neto et al. 2017). They also enhance the photosynthetic pigments which finally improves photosynthetic efficiency of the plant under stress conditions. Sharma et al. (2016) reported that exogenous application of 24-epibrassinolide (Ebl) mitigated the heavy metal toxicity in *Oryza sativa* L. by enhancing enzymatic activities, nonenzymatic antioxidants, and pigment contents. Ebl treatment also enhances the gene expression of defense system in various plants to increase their tolerance under heavy metal stress (Sharma et al. 2013) (Table 2).

3.4 Role of Ethylene in Heavy Metal Toxicity Mitigation

Ethylene, a gaseous hormone, has multitude roles in plants and controls morphological and photosynthesis parameters under normal and stressful environment. It has the potential role in enhancing plant tolerance to heavy metal stress. Plants producing more ethylene are reported to have higher resistance against heavy metal stress (Ashger et al. 2018; Keunen et al. 2016). Exogenous application of ethylene significantly lowered the Cd concentration in *Catharanthus roseus*. MDA and H_2O_2 production was also reduced in roots and leaves, signifying that the ethylene application effectively alleviated the Cd stress (Chen et al. 2017). It is also observed that ethylene reversed the inhibition of elongation of primary root induced by Cr (VI) in *Arabidopsis thaliana.* Ethylene stimulates the expression of AUXIN-RESISTANT (AUX 1) and alleviates Cr (VI) toxicity by enhancing the accumulation of auxin and its polar transport (Wakeel et al. 2018). The role of ethylene in mitigating heavy metal toxicity and in signaling pathways is well documented. It is reported that EIN2 is implicated in the ethylene signaling pathway and transduction in response to stress. EIN2 transcript levels are enhanced by heavy metals which stimulate AtPDR12 that prevents the accumulation of toxic metals in cytoplasm (Bücker-Neto et al. 2017).

3.5 Role of Cytokinins in Heavy Metal Toxicity Mitigation

Cytokinins are forms of plant hormones and adenine metabolites which divide and differentiate plant cells, promote bud formation and blooming, generate lateral buds and leaves, and synthesize chlorophyll (Werner et al. 2001). Cytokinin slows plant ageing by gathering chlorophyll, transforming etioplast into chloroplasts, and neutralizing reactive oxygen species. It can then adapt to environmentally sustainable circumstances of the plant (Ashraf et al. 2008; Grossman and Leshem 1978).

Table 2 Role of brassinosteroids in heavy metal toxicity amelioration

S. no.	Brassinosteroid	Heavy metal	Plant name	Effects	References
1.	Epibrassinolide	Sb	*Arabidopsis thaliana*	Decreased the uptake of Sb and peroxidation of membrane lipids, enhanced contents of proline and chlorophyll, and improved antioxidant enzyme activities	Wu et al. (2019)
2.	24-Epibrassinolide	Cu	*Vitis vinifera*	Improved root growth parameters, enhanced content of proline and soluble protein, reduced malondialdehyde, H_2O_2, and O_2^{-} contents in roots and leaves Modulated activities of enzymatic antioxidants. Enhanced content of salicylic acid, abscisic acid, and jasmonic acid in the leaves	Zhou et al. (2018)
3.	24-Epibrassinolide and 28-homobrassinolide	Cr or Cd	*Raphanus sativus*	Upregulation of antioxidant enzymes (MnSod, FeSod, Cu/ZnSod, Cat1, Cat2, Cat3)	Sharma et al. (2018)
4.	24-Epibrassinolide and silicon	Cd	*Pisum sativum*	Modulated antioxidant defense. Improved morphological and biochemical parameters, enhanced glyoxalase I level, improved osmolyte content, reduced Cd accumulation	Jan et al. (2018)
5.	Castasterone and citric acid	Cd	*Brassica juncea*	Increased content of total carbohydrates, organic acids, and phenolic compounds. Enhanced chlorophyll and carotenoid content	Kaur et al. (2018)
6.	24-Epibrassinolide	Cd	*Vigna unguiculata*	Reduced transport of Cd, increased photosynthetic pigments, reduced MDA content, increased content of essential elements	Santos et al. (2018)
7.	24-Epibrassinolide and salicylic acid	Pb	*Brassica juncea*	Restored growth, increased concentration of lipid-soluble and total water antioxidants, photosynthetic pigments, enhanced content of nonenzymatic antioxidants	Kohli et al. (2018)

(continued)

Table 2 (continued)

S. no.	Brassinosteroid	Heavy metal	Plant name	Effects	References
8.	Epibrassinolide	Cd	Tomato	Restored growth, enhanced photosynthetic pigments, reduced accumulation of Cd in tomato	Guo et al. (2018)
9.	Castasterone and citric acid	Cd	*Brassica juncea*	Improved growth attributes, increased Cd uptake by roots, decreased H_2O_2 and increased NO content, enhanced GSH content, enhanced activities of antioxidant enzymes	Kaur et al. (2017)
10.	Epibrassinolide and selenium	Cu	*Brassica juncea*	Enhanced photosynthesis, modulated activities of various antioxidant enzymes, increased proline content	Yusuf et al. (2016)
11.	24-Epibrassinolide	Cr	Tobacco	Improved growth parameters and enhanced photosynthesis, reduced oxidative stress, and decreased uptake in different parts	Bhukhari et al. (2016)
12.	24-Epibrassinolide	Cr	*Oryza sativa*	Enhanced expression of antioxidant enzymes Mn-SOD, Cu/Zn-SOD, CAT A, CAT B, APX, and GR; reduced Cr uptake; and improved seedling growth	Sharma et al. (2014)
13.	24-Epibrassinolide and 28-homobrassinolide	Zn	*Raphanus sativus*	Improved growth attributes and relative water content; enhanced content of photosynthetic pigments; enhanced activities of superoxidase dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX); and reduced activities of glutathione reductase (GR) and peroxidase (POD). Increased contents of proline, glutathione (GSH), and ascorbate (ASA)	Ramakrishna and Rao (2015)
14.	28-Homobrassinolide	Cd and Hg	*Raphanus sativus*	Elevated protein content, enhanced activities of antioxidant enzymes	Sharma et al. (2014)

(continued)

Table 2 (continued)

S. no.	Brassinosteroid	Heavy metal	Plant name	Effects	References
15.	28-Homobrassinolide	Cd	*Triticum aestivum*	Improved growth, increased photosynthetic pigments, reduced MDA and H_2O_2. Increased proline content, enhanced activities of antioxidative enzymes	Hayat et al. (2014)
16.	24-Epibrassinolide	Cu	*Cucumis sativus*	Restored growth, chlorophyll content, enhanced activity of carbonic anhydrase, improved photosynthetic efficiency, increased proline content, and enhanced activities of various antioxidant enzymes, viz. superoxide dismutase (SOD), catalase (CAT), peroxidase (POD)	Fariduddin et al. (2013)

Research studies showed that the use of this hormone has enhanced the transpiration and therefore also enhanced heavy metal accumulation in the solution (Tassi et al. 2008). Plant hormones were used by Cassina et al. to improve metal extraction in *Alyssum murale*. Moreover, it was also observed that cytokinin use on *Alyssum murale* increased the amount of aerial biomass relative to control (Cassina et al. 2011). Tassi et al. studied the use of cytokinin on *Helianthus annuus* and its role in the adsorption of heavy metals like lead and zinc. The analysis indicates that cytokinin's application significantly improved plant productivity and metal absorption in the aerial portions of plants. The improvement in the productivity of plants might be due to an enhancement in the division of cells. Studies have shown that enhancement in stomatal transpiration and stroke conduction after treatment with IAA and cytokinin may increase the mechanism of heavy metal adsorption and transfer (Cassina et al. 2011). However, cytokinins interact with plant metal uptake pathways that may improve glutathione levels and alter metallothionein expression, ultimately improving plant tolerance to HMs (Thomas et al. 2005). Kinetin induces cell division and plant growth at high concentrations. Moreover, increase in cell count thus improves the root absorption efficiency. Cytokinins have also been reported to enhance photosynthesis and photosynthetic compounds, which may improve the transition of one cell to another cell (Sayed 1999).

3.6 Role of Jasmonates in Heavy Metal Toxicity Mitigation

Jasmonic acid is an oxylipin-based plant hormone quickly synthesized in response to various abiotic and biotic stresses (Vleesschauwer et al. 2013). Various reports suggested that JA (jasmonic acid) signaling and biosynthesis separately regulate the responses and adaptation of plants under abiotic stresses (Du et al. 2013) such as heavy metals and salinity (Qiu et al. 2014; Keramat et al. 2009). Another class of cyclopentanone is MeJA (methyl jasmonate) that plays an important role to ameliorate the effect of various environmental stresses (Santino et al. 2013). The exogenous appliance of JA at low concentrations has been involved in plant growth and tolerance against abiotic stresses (Cheong and Choi 2003; Chen et al. 2014a, b, c). Various effects on plants under different metals with jasmonic acid are shown in Table 3.

3.7 Role of Salicylic Acid in Heavy Metal Toxicity Mitigation

A phenolic compound known as salicylic acid (SA) is a phytohormone which is involved in the regulation of the growth and development processes, senescence, and other metabolic processes (Vicente and Plasencia 2011). Gondor et al. (2016) have reported that exogenous application of SA helps in increasing abiotic stress tolerance. Heavy metal toxicity causes an inhibitory effect in the plant growth and development (Yang et al. 2015; Zengin 2014). Various reports on exogenous application of SA involve the reduction and mitigation of heavy metals on plants (Han et al. 2015; Mingxuan et al. 2015). Several effects on plants under different metals with salicylic acid are shown in Table 4.

3.8 Role of Gibberellins in Heavy Metal Toxicity Mitigation

Gibberellins were originally discovered as a fungal toxin causing unusual shoot elongation of rice plants (Yamaguchi 2008). It belongs to the family of tetracyclic diterpenoid plant growth substances (Sponsel and Hedden 2004) which couple with various physiological activities like seed germination, leaf expansion, floral initiation, floral organ development, and induction of some hydrolytic enzymes. It has also been accounted that gibberellins also play a major role in modulating the plant growth and development under various environmental stresses (Iqbal et al. 2011; Shukla et al. 2017). Several studies revealed that GA alleviates various abiotic stresses including heavy metal toxicity. He et al. (2012) observed the role of GAs in increasing stress tolerance by enhancing the expression of the TaMYB73 gene in wheat. DELLA protein, a repressor of GA responses, has recently been shown to be involved in stress avoidance (Wild and Achard 2013). It was also found that a low

Table 3 Application of jasmonic acid in heavy metal toxicity amelioration

S. no.	Plant species	Heavy metal stress	Exogenous hormonal treatment (name and concentration)	Effects on plant	References
1.	*Vicia faba*	Cd	JA	Increases water content and improves root and shoot lengths	Ahmad et al. (2017)
2.	*Solanum lycopersicum*	Pb	JA exogenous	Improves the chlorophyll and carotenoid contents	Bali et al. (2018)
3.	*Wolffia arrhiza*	Pb	JA (0.1 µM)	Restores carotenoid level and inhibits Pb accumulation	Piotrowska et al. (2009)
4.	*Brassica napus*	As	MeJA 0.1 and 1 µM	Increases biomass	Farooq et al. (2016)
5.	*Solanum lycopersicum*	Pb	JA (0.1 µM)	Improves growth, decreases the uptake of metal	Bali et al. (2018, 2019)
6.	*Arabidopsis thaliana*	Cu and Cd	MeJA	Stimulates the accumulation of phytochelatins	Maksymiec et al. (2007)
7.	*Oryza sativa*	Cd	MeJA	Reduces MDA and H_2O_2	Singh and Shah (2014)
8.	*Glycine max*	Ni	JA	Increases transcript levels of POD and CAT	Sirhindi et al. (2016)
9.	*Brassica napus*	Cd	Exogenous JA	Repairs damage	Ali et al. (2018)
10.	*Glycine max*	Pb	JA	Protective effects during photosynthesis	Keramat et al. (2009)
11.	*Kandelia obovata*	Cd	MeJA 0.1–10 mmol L^{-1} exogenous	Reduces the translocation of Cd from roots to leaves	Chen et al. (2014a, b, c)
12.	*Oryza sativa*	Cd	MJ at 5 µM	Ameliorated effect	Singh and Shah (2014)
14.	*Solanum nigrum L.*	Cd	MeJA 0.1 µM	Enhanced metal tolerance	Yan et al. (2015a, b)
15.	*Capsicum frutescens*	Cd	MeJA 0.1–1 µM	Enhanced metal tolerance	Yan et al. (2013)
16.	*Cajanus cajan*	Cu	JA	Increases proline (proline has antioxidant property)	Sharma et al. (2013)
17.	*Avicennia marina*	Cd	1–10 µmol L^{-1} of JA	Translocation of Cd from roots to leaves is reduced	Yan et al. (2015a, b)
18.	*Pisum sativum L.*	Cr	Exogenous JA	Reduced Cr uptake	Gangwar and Singh (2011)

Table 4 Application of jasmonic acid in heavy metal toxicity amelioration

S. no.	Plant species	Heavy metal stress	Exogenous hormonal treatment (name and concentration)	Effects on plant	References
1.	*Triticum aestivum*	Cd	Exogenous SA	Increased levels of relative water content in leaf tissues	Agami and Mohamed (2013)
2.	*Phaseolus vulgaris*	Cd	Exogenous SA	Increased levels of relative water content in leaf tissues	Wael et al. (2015)
3.	*Solanum tuberosum*	Cd	Foliar application of SA	Increased levels of relative water content in leaf tissues	Li et al. (2019)
4.	*Lemna minor*	Cd	Exogenous SA	Increased SOD activity	Lu et al. (2018)
5.	*Poa pratensis*	Cd	Exogenous SA	Increased SOD activity	Guo et al. (2013)
6.	*Oryza sativa*	Cd	Exogenous SA	Increased SOD, APX, and GR activity	Chao et al. (2010)
7.	*Oryza sativa*	Pb	SA	Increased chlorophyll content	Jing et al. (2007)
8.	*Hordeum vulgare*	Cd	SA	Prevents toxicity	Metwally et al. (2003)
9.	*Helianthus annuus L*	Cu	SA	Increases tolerance	El-Tayeb et al. (2005)
10.	*Pisum sativum*	Pb	SA	Improves the growth of plant	Ghani et al. (2015)
11.	*Sorghum bicolor L.*	Pb	SA	Increases growth	Sihag et al. (2019)
12.	*Arabidopsis thaliana*	Pb	Exogenous SA	Increases length and biomass of shoots and roots	Vanacker et al. (2001)
13.	*Cassia tora*	Al	SA	Reduces the accumulation and translocation of aluminum	Yang et al. (2003)
14.	*Vallisneria natans*	Pb	SA	Reduces accumulation	Wang et al. (2012)
15.	*Zea mays and Pisum sativum*	Cd	SA	Alleviates harmful effects	Krantev et al. (2008); Popova et al. (2009)
16.	*Oryza sativa* seedlings	Cd	Sulfo-SA	Decreases the accumulation of Cd in roots and shoots	Singh et al. (2015)
17.	*Solanum lycopersicum*	Cd	SA	Improves leaf growth and fresh weight	Çanakci (2012)

(continued)

Table 4 (continued)

S. no.	Plant species	Heavy metal stress	Exogenous hormonal treatment (name and concentration)	Effects on plant	References
18.	*Hordeum vulgare*	Cd	SA	Promotes barley seed germination and early seedling growth	Kalai et al. (2016)
19.	*Hordeum vulgare*	Cd	SA	Prevents oxidative stress and lipid peroxidation	Pal et al. (2002)
20.	*Oryza sativa*	Pb	Exogenous SA 100 μM	Boosts growth and photosynthetic	Chen et al. (2017)
21.	*Zea mays*	Cd	Exogenous SA 500 μM	Boosts growth and photosynthetic	Krantev et al. (2008)
22.	*Phaseolus vulgaris*	Cu	Exogenous SA	Boosts growth and photosynthetic	Zengin (2014)
23.	*Pisum sativum*	Cd	Exogenous SA	Protects Rubisco activity against Cd damage	Popova et al. (2009)

concentration of heavy metal (Zn) is able to increase the content of GA_3; however higher concentrations decrease the GA_3 level (Atici et al. 2005). Similarly, GA plays a crucial role in providing protection against Cd stress by suppressing the expression of iron-regulated transporter 1 (IRT1), a transporter that is involved in Cd uptake in *Arabidopsis thaliana* (Zhu et al. 2012) (Fig. 1). In *Brassica napus* also, GA reduced Cd-induced adverse effects on seed germination and growth by regulating oxidative stress and ROS damage (Meng et al. 2009). This hormone also eliminated the effects of Cd and Pb by boosting the activities of proteases, catalases, and peroxidases in broad bean and lupin plants (Sharaf et al. 2009). In this parallel line, Khan and Lee (Khan and Lee 2013) determined endophyte-metal-plant interaction with *Penicillium funiculosum*-Cu-*Glycine max* and observed significant amelioration of Cu toxicity in endophyte-inoculated plants with increased biomass. The positive effects of endophyte were due to gibberellin secretion and decline in stress-induced ABA levels. Similarly, the effectiveness of endophyte *Penicillium janthinellum* or GA_3 application on aluminum (Al) tolerance was studied by Khan et al. (2015) and found similar possible application of endophyte and exogenous GA_3. For *Arabidopsis*, it has been confirmed that the expression of adenosine-5-phosphosulfate reductase (APR), the key enzyme of sulfate assimilation, is increased using GA signaling under stress. This suggests that GA-mediated signaling may be utilized under metal stress to improve sulfur metabolism. Application of GAs regulated enzymatic activities in nitrogen assimilation with further reduction of nitric oxide accumulation (Gangwar et al. 2011; Zhu et al. 2012). GAs inhibited iron (Fe) translocation by suppressing OsYSL2 gene expression in addition to regulation of Fe transport and translocation (Wang et al. 2017a, b).

Furthermore, authors observed that GA reduces NO level which in turn also downregulates the expression of IRT1 gene (a Fe transporter might be involved in

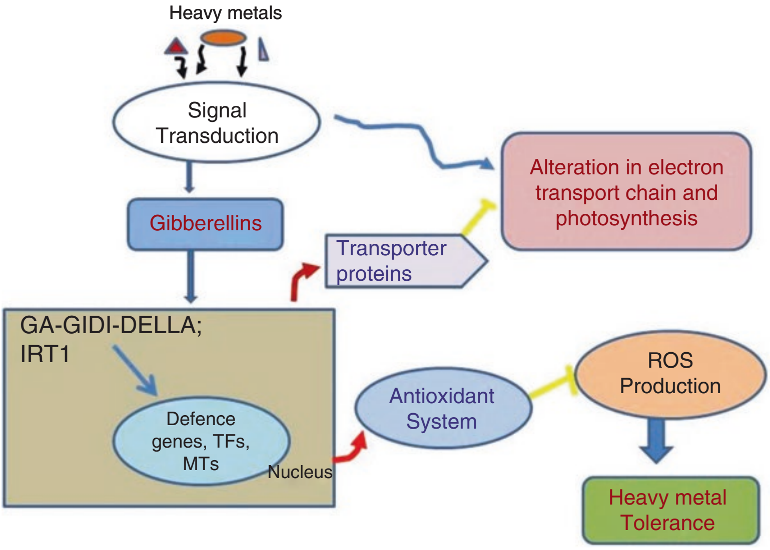

Fig. 1 Gibberellins mediated signaling and heavy metal tolerance in plants by signal cascade of different enzyme expression

Cd absorption). They confirmed this as indicated by no effect of GA being reported in the reduction of Cd uptake in an IRT1 knockout mutant irt1. Therefore through the above studies, it concludes that gibberellins may regulate heavy metal stress in plants through alteration in ROS and RNS levels, expression of antioxidant enzymes, metal transporters, as well as sulfur metabolism (Sytar et al. 2019a, b) (Fig. 1). These studies clearly indicate that GA plays an important role in protecting plant metabolism against various stresses.

4 Cross Talk of Plant Growth Regulators Under Heavy Metal Toxicity

Plant growth regulators are small signaling compounds that usually act at nanomolar concentrations on every aspect of plant growth and development. Hormones build a signaling network and regulate several signaling and metabolic systems, which are essential for plant development and plant responses to biotic and abiotic stresses. Although earlier work greatly advanced our knowledge of how hormones affect plant growth and development and stress responses, it is now evident that physiological processes are regulated in a complex way by the cross talk of several hormones (Nishiyama et al. 2011; Munne-Bosch and Muller 2013; Colebrook et al. 2014; Xu et al. 2016a, b; Bucker-Neto et al. 2017). Phytohormones act as regulators of heavy metal absorption in agronomical crop management practices to alleviate metal toxicity (Piotrowska-Niczyporuk et al. 2012) and they have significant roles in signaling and defense pathways (Bucker-Neto et al. 2017). There are several studies showing the involvement of phytohormones in remodeling the architecture in response to heavy metal stress (De Smet et al. 2015; Jalmi et al. 2018a, b).

Auxin is one of the essential plant growth hormones playing a role in developmental as well as environmental stress responses. It affects plant responses to metal stresses by amending auxin homeostasis (Potters et al. 2007; Jalmi et al. 2018a, b). Yuan et al. (2013) have reported that excess Cu affects both elongation and meristem zones by altering auxin distribution through PINFORMED1 *(PIN1)* protein and is responsible for inhibition of primary root elongation under the influence of Cu-mediated auxin redistribution. Similarly, excess Cu inhibits root length and alters morphology by inducing alterations in auxin levels, which antagonizes the effect of nitric oxide function (Peto et al. (2011). This indicates that alteration in hormonal balance correlates with heavy metal-mediated toxicities in plants (Peto et al. 2011; Wilkinson et al. 2012). For example, in *Brassica juncea*, As causes toxicity by changing the levels of the auxin:indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) and altering the expression of microRNAs (Srivastava et al. 2013). However, exogenous supply of IAA improves the growth of *B. juncea* under As stress, suggesting an implication of the regulation of the hormone level in the management of As stress. The increased IAA level has been associated with growth reduction, which can be a result of changed hormonal balance under stress conditions (Fahad et al. 2015). The hormone interaction and miRNA expression in the regulation of HM (As) response have been suggested under exogenous supply of IAA. It was shown to improve the growth of plants under HM (As) stress (Srivastava et al. 2013). IAA, ROS, and ET cross talk and cooperate during the signaling pathway with alteration in the root system of plants (Camacho-Cristobal et al. 2015).

The *in vivo* importance of auxin and cytokinin antagonistic interaction in root patterning and organogenesis has been deeply studied using *A. thaliana* model plant (Werner and Schmulling 2009; Pacifici et al. 2015; Chaiwanon et al. 2016; Slovak et al. 2016). Cytokinins play a regulatory role in modulating plant development and their endogenous concentration under stress conditions, which indicates the involvement of CKs in stress tolerance (Brien and Benkova 2013). HM stress decreases CK production and transport from roots. CKs during HM stress participate intensively in interactions with other hormones (Ha et al. 2012) and often they are antagonists of ABA (Pospisilova 2003). Thus changes in the levels of both plant hormones under HM stress can be dependent on each other as a result of their cross talk (Sytar et al. 2019a, b). Moreover and for the first time Cd toxicity in root growth and pattern has been related to a mis-expression of SCR transcription factors which is known to interplay with auxin/cytokinin cross talk in the control of RAM maintenance and activity (Bruno et al. 2017).

Kang et al. (2017) investigated the IAA and GA production ability for rhizospheric bacterium *Leifsonia xyli* SE134 which could increase the nutrient content in tomato. They confirm the ability of gibberellic acid/IAA in exogenous form and microbial GAs/IAA to promote the uptake of Fe and P and subsequently decrease

Cu stress. Therefore, the synchronization of all these growth-promoting activities of *L. xyli* SE134 increased the survival and tolerance of tomato plants under extreme conditions of Cu heavy metal stress.

Literature analysis had shown that IAAs, CKs, and GAs positively affect the level of metal accumulation and improve plant growth and tolerance to the stress (Bulak et al. 2014). In rice crop, heavy metals such as cadmium (Cd) and arsenic (As) induced harmful effects by altering growth, metabolism, and productivity (Zanella et al. 2016; Fattorini et al. 2017; Bruno et al. 2017; Ronzan et al. 2019). Ronzan et al.'s (2019) results showed that JAs reduce the damages induced by As and/or Cd in rice depending on the root type. But when JAs interact with auxin, it affected its homeostasis and reduced the effects of the two pollutants. These changes minimize complex interactions among them. JAs mediate the expression of IAA biosynthetic genes, as ANTHRANILATE SYNTHASE-alpha1 (ASA1) and members of the YUCCA family (YUCCA6, YUCCA8, and YUCCA9), all involved in root growth (Sun et al. 2009; Hentrich et al. 2013; Veloccia et al. 2016; Fattorini et al. 2017). So far, the cross talk between auxin and JAs in the presence of As and Cd has been poorly investigated in rice root development. However, OsASA2 and OsYUCCA2 genes have been observed to be active in the biosynthesis of IAA and affected by Cd and/or As. These actions may be due to complex interactions among phytohormones, e.g., auxin and jasmonates (JAs), and caused the synthesis of various types of stressors, e.g., reactive oxygen species (ROS), affecting the expression of numerous genes related to the plant ability to respond to and defend metal/metalloid toxicity.

Abscisic acid hormone level in plants is tightly controlled by various environmental conditions. The high level of ABA activates signaling cascades of other phytohormones, such as salicylic acid (SA) and jasmonic acid (JA) (Shi et al. 2015), under heavy metal stress. A study by Shi and Gibson (2011) recommended that exogenous application of ABA can decrease the phytotoxic effect of Zn in *Populus canescens* by modulating the transcriptional key genes involved in Zn transport and detoxification. Ahmad et al. (2015) reported that the ABA and GA_3 hormone's synergistic interaction with Pb and Zn caused an increase in total phenolic content; however interaction with Cd puts off an increase in phenolic compounds. Thus the effect of HMs alone or in combination with phytohormones has different influences on plants, depending on the type of plant, their development stage, HM concentration, and duration of treatment. It was found that prolonged Pb treatment together with GA_3 influenced the soluble protein content in a positive manner (Nuray and Işil 2012). Exogenous application of IAA, GA_3, and citric acid increased plant biomass (Aderholt et al. 2017). Application of GAs regulated enzymatic activities in nitrogen assimilation with further reduction of nitric oxide accumulation (Gangwar et al. 2011; Zhu et al. 2012). GAs inhibited iron (Fe) translocation by suppressing OsYSL2 gene expression in addition to regulation of Fe transport and translocation (Wang et al. 2017a, b).

Ethylene and its cross talk with other phytohormones or with signaling molecules are important for plant adaptation under HM-induced stress (Thapa et al. 2012; Montero-Palmero et al. 2014a, b). It has been found that upon exposure to stress, the levels of jasmonic acid (JA), salicylic acid (SA), abscisic acid, and ethylene increase, while the contents of GA_3 and auxin decrease in plants (Metwally et al. 2003; Canovas et al. 2004; Atici et al. 2005; Maksymiec et al. 2005). It was observed that Al treatment in *Arabidopsis* led to the increased expression of ethylene biosynthesis-related genes (Sun et al. 2010). However, in wild-type plants, Al treatment also enhanced the transcript of AUXIN RESISTANT1 (AtAUX1) and PINFORMED2 (AtPIN2), but ethylene synthesis and perception inhibitors (Co and AVG) brought down Al-induced expression of AtAUX1 and AtPIN2. These data showed that auxin redistribution affected Al-induced ethylene production by affecting auxin polar transport systems through AUX1 and PIN2 (Sun et al. 2010), which is an indicator of possible cross talk between ethylene and auxin in plant responses to HM stress.

Furthermore, the study of Yuan et al. (2013) also observed that PIN2 and AUX1 and wild-type plants exhibited similar effects on the inhibition of primary root elongation under Cu stress. Their results indicated that ethylene-mediated signaling is not required for the Cu-inhibited primary root elongation. However, these findings indicated that genes involved in the control of auxin redistribution might be specific, and act dependently or independently of ethylene. Their studies confirmed that the enzymes responsible for auxin biosynthesis (*ASA1/WEI2/TIR7, ASB1/WEI7, TAA1/SAV3/WEI8*), auxin-responsive factors (*ARF2, ARF1*), and auxin transporters (*PIN1, PIN2, PIN4, AUX1*) are the genes which are regulated by ethylene.

In another study, it was revealed that the ethylene and JA signaling pathways stabilized transcription factors (EIN3 and EIL1) and function synergistically and independently in the regulation of gene expression in *Arabidopsis* (Zhu et al. 2011; Van der Does et al. 2013). Under prolonged Cd stress in *Arabidopsis* plants, these two hormone signaling pathways were activated, leading to the upregulation of NITRATE TRANSPORTER1.8 (*NRT1.8*) and the downregulation of *NRT1.5*, which mediated the stress-initiated nitrate allocation to roots to enhance the tolerance to Cd stress (Zhang et al. 2014). JAs can neutralize toxic effects of low concentrations of Cu and Cd by inducing the accumulation of phytochelatins, glutathione, and carotenoids, which results in an enhanced plant tolerance. In pea plants, increased JAs and ethylene, together with ROS, regulate the induction of pathogenesis-related proteins that protect the plant from Cd-related damages (Rodriguez-Serrano 2009).

A naturally occurring phenolic compound, SA, is also linked to the defense response of plants under HM stress. In the SA signaling, several signaling molecules such as nitric oxide (NO), H_2O_2, and Ca^{+2} and their interactions have been reported under excessive Cd (Rodriguez-Serrano et al. 2009; Xu et al. 2015). Moreover, Cui et al. (2012) have reported a cross talk of heme oxygenase-1 and SA in alleviation of Cd stress in *M. sativa*. SA often acts in combination with other phytohormones such as JA and ET (Jia et al. 2013). The biosynthesis of hormones and their transport and accumulation generate a cascade of signaling pathways, which are part of the plant stress response (Matilla-Vazquez and Matilla 2014). The activity of ET biosynthetic enzymes increases MAPK phosphorylation of ACS2 and ACS6 (Skottke et al. 2011; Bucker-Neto et al. 2017).

Masood and Khan (2013) studied that treatment with GA_3 and sulfur (S) at optimal concentration reduced undesirable stress ethylene induction, resulting in the alleviation of photosynthetic inhibition caused by Cd stress. It is well known that S assimilation leads to Cys biosynthesis, which is required for both ethylene and GSH biosyntheses under normal conditions (De Grauwe et al. 2008; Iqbal et al. 2013). Under HM stress, application of S to Cd-treated plants was noticed to adjust stress-induced ethylene content to an optimized level, which afterward led to a maximal GSH content, thereby providing effective protection again oxidative stress and, thus, alleviating Cd-induced toxicity in plants (Asgher et al. 2015). This suggested the role of the GSH pathway in the mitigation of HM stress through ethylene and ethylene signaling that might also involve the S pathway. On the other hand, there was also evidence that the EIN2 gene mediates Pb resistance *Arabidopsis* plants through PDR12, which is a member of the ATP-binding cassette transporter G family and is induced by auxin, abscisic acid, ethylene, JA, and SA (Shukla et al. 2014). In response to HMs, not only ethylene but also other hormones, including brassinosteroids, auxin, SA, GA_3, and cytokinin, were shown to stimulate the antioxidant responses in order to scavenge different ROS when plants were grown under Cd, Cu, or Pb stress (Hayat et al. 2007; Noriega et al. 2012; Piotrowska Niczyporuk et al. 2012). SA treatment increased the GSH content and resulted in an induction of antioxidant and metal detoxification systems, which led to Cd stress tolerance in wheat (*Triticum aestivum*) and pea (*Pisum sativum*) as well as amelioration of the negative effects of Cu stress in *Brassica napus* (Khademi et al. 2014; Kovacs et al. 2014).

Strigolactone and ABA are both derived from carotenoids and their biosynthesis genes are closely related to each other. The ABA transporter is homologue to SL transporter in *Arabidopsis*. Thus the biosynthesis and transport of these hormones are closely interdependent. Because of the close relation with ABA, SLs may also be affected when ABA biosynthesis or transport is affected by metal stress. The involvement of SLs in root hair elongation could also be important during drought responses.

Brassinosteroids are the first plant steroids with a regulatory nature. Fu et al. reported that they played a regulating role in the plant by stimulating the signaling networks of other plant hormones (Fu et al. 2008). Several studies have also shown that brassinosteroids are able to increase performance in several plant species, and the results depend on the usage, plant growth stages during usage, and environmental conditions (Divi et al. 2010; Rostami and Azhdarpoor 2019).

Brassinosteroids, a polyhydroxy steroidal compound, carry out diverse functions due to its interplay with other phytohormones. BRs interact with different phytohormones such as abscisic acid (ABA), auxin, cytokinin (CK), ethylene, gibberellins (GA), jasmonic acid (JA), polyamines (PA), and salicylic acid (SA) in response to environmental cue and regulate numerous aspects of plant growth and developmental processes in plants (Choudhary et al. 2012; Gruszka 2013).

The application of 24-EBL and Si, individually and in combination, alleviated the adverse effects of Cd by improving growth, biochemical parameters, nutrient uptake, osmolyte accumulation, and antioxidative defense and glyoxalase systems in *Pisum sativum* seedlings (Jan et al. 2018). BR stimulates ethylene production by stabilizing ethylene biosynthesis enzymes ACS5 and ACS9. Note that cytokinin had the same effect on ACS5, ACS9, and thus ethylene production (Schellingen et al. 2014). Thus, both BRs and cytokinins contribute to the ethylene-auxin cross talk that controls cell elongation, by stimulating ethylene production. In *Brassica napus* and *Lycopersicon esculentum*, BRs reduce the toxic effects of Cd on phytochemical processes by diminishing the damage on photochemical reaction centers and the activity of oxygen-evolving center as well as by maintaining efficient photosynthetic electron transport. However, in *A. thaliana* BRs do not seem to have the protective effect to Cd stress. Cd exposure triggers the activation of the BR signaling pathway and high BR contents lead to hypersensitivity to Cd (Schellingen et al. 2014).

Hence, environmental stresses require plants to perceive and react to these signals in a highly coordinated and interactive manner. Plants being sessile organisms need to maintain plasticity in growth and ability to adapt to harsh changing environmental conditions, and this adaptation is mediated by elaborate signaling networks by phytohormones with the perception of abiotic stresses. Exogenous application of CKs, IAAs, GAs, SA, ET, BRs, and SLs can increase the level of antioxidants and stimulate plant growth. Thus, plants may survive better with phytohormone priming in HM contamination and thus play a central role in coordinately regulating growth responses under stress conditions (Table 5).

The elicited physiological processes are dependent not only on the perceived stimulus, but also on the specific properties of tissue to a given signaling molecule class. Therefore the plant hormone network can affect plant development and physiological action largely respond on several different levels involving control of gene expression (mRNA and protein synthesis), configuration, modification, as well as hormone transport and reversible or irreversible inactivation of active signaling molecules.

5 Conclusion

Heavy metal toxicity is a serious threat to the global agriculture system. Plants being a part of sessile habit induce some structural, physiological, and molecular mechanisms to cope up with such abiotic stressors. Scientific community has recognized the potential role of PGRs in the mitigation of heavy metal stress. Exogenous phytohormonal treatment to various crops has shown the potential role of these hormones in the amelioration of heavy metal toxicity. But still there are multiple cross talks which are required to be discovered.

Table 5 Cross talk of plant growth regulators under heavy metal toxicity

S. no.	Plant name	Hormonal cross talk	Heavy metal alleviated	References
1.	*Pisum sativum*	24-Epibrassinolide and silicon	Cd	Jan et al. (2018)
		IAA-Asp (an auxin conjugate) affects pea responses to Cd by modulating antioxidative defense response	Cd	Ostrowski et al. (2016)
2.	*Brassica juncea L.*	Epibrassinolide induces changes in indole-3-acetic acid, abscisic acid, and polyamine concentrations and enhances antioxidant potential	Cu	Choudhary et al. (2010)
		Ethylene and gibberellic acid	Cd	Masood and Khan (2013)
		Co-application of 24-epibrassinolide and salicylic acid improving growth, contents of pigment of metal-stressed plants. Glutathione, ascorbic acid, and tocopherol contents were significantly enhanced	Pb	Kohli et al. (2017, 2018)
3.	*Arabidopsis thaliana*	Antagonistic interaction between auxin and CK in their relationship with PCs	Cadmium	Pacifici et al. (2015); Chaiwanon et al. (2016); Slovak et al. (2016)
		Ethylene and auxin Al-induced ethylene production may lead to auxin redistribution by affecting auxin polar transport systems through AUX1 and PIN2	Aluminum (Al) Cu	Sun et al. (2010); Yuan et al. (2013, b)
		Boron deficiency inhibits root cell elongation via an ethylene/auxin/ROS-dependent pathway in *Arabidopsis* seedlings	Boron	Camacho-Cristobal et al. (2015)
		Ethylene and JA signaling	Cd	Zhu et al. (2011); Van der Does et al. (2013); Zhang et al. (2014)
		Ethylene and GA_3	Cd	Masood and Khan (2013)
		Ethylene and GSH pathway	Pb	Iqbal et al. (2013)

(continued)

Table 5 (continued)

S. no.	Plant name	Hormonal cross talk	Heavy metal alleviated	References
		Auxin and cytokinin antagonistic interaction		Pacifici et al. (2015); Chaiwanon et al. (2016); Slovak et al. (2016)
		Auxin, abscisic acid, ethylene, JA, and SA upregulated in Arabidopsis plants treated with AuCl24	AuCl	Shukla et al. (2014)
		Brassinosteroids, auxin, SA, GA3, and cytokinin	Cd, Cu, or Pb stress stimulates the antioxidant responses	Noriega et al. (2012); Piotrowska et al. (2012)
4.	*Oryza sativa*	Auxin-jasmonate An auxin/jasmonate interaction affects rice root system development in the presence of cadmium and/or arsenic, even if exogenous jasmonic acid methyl ester only slightly mitigates pollutant toxicity	Cadmium and/or arsenic	Ronzan et al. (2019)
		Co-application of selenium (Se) and auxin; Se and auxin were more effective in reducing As-induced stress compared to individual treatments	As	Pandey and Gupta (2015)
5.	*Tomato*	Gibberellins and indole-3-acetic acid The synchronization of these growth-promoting activities of *L. xyli* SE134 increased tolerance of tomato plants under Cu heavy metal stress	Cu	Kang et al. (2017)
6.	*Hordeum vulgare*	Salicylic acid and auxin mediated SA probably does not alleviate the Cd toxicity in roots, but prevents defense to Cd through the inhibition of Cd-induced IAA-mediated ROS generation in roots	Cadmium	Tamas et al. (2015)

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Heavy Metal Mitigation with Special Reference to Bioremediation by Mixotrophic Algae-Bacterial Protocooperation

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1 Introduction

The phrase “heavy metal” denotes a natural element with relatively higher density than water. The various heavy and transition metals, such as Cd^{2+}, Cr^{2+}/Cr^{3+}, Cu^{2+}, F^{2+}/ Fe^{3+}, Hg^{2+}, Ni^{2+}/Ni^{4+}, Zn^{2+}, and Cu^{2+} (Tchounwou et al. 2012; Lohar et al. 2013; Banik et al. 2018; Mondal et al. 2019), have been grouped as essential and nonessential based on its biological functions. Essential metals are important constituents of various enzymes involved in physiological functions of living organisms and are required at very low concentrations. Any slight increase in concentration affects the vital biological processes, e.g., Fe, Na, K, Ca, Mg, Zn, Mn, Co, Ni, Mo, and Cu (Filali et al. 2000; Naskar et al. 2016). In contrast, the nonessential metals have unidentified biological roles and are very noxious at very low concentrations, e.g., Hg, Pb, Cd, Cr, Ag, Sn, Au, Ti, Al, Ge, As, Sb, Se, Rb, Cs, Sr etc. (Roane and Pepper 2000; Mahapatra et al. 2013; Banik et al. 2018).

Industrialization, intensive agriculture, urbanization, and anthropogenic activities are the main sources of contamination of different environmental bodies with toxic heavy metals and various organic pollutants (Jaishankar et al. 2014). The metals are nonbiodegradable by nature and tend to bioaccumulate. The accumulation and biomagnification of toxic metals in living organism facilitate their entry into food chain (Ali et al. 2013) and cause toxicity by displacing vital metal ions from cellular macromolecules, by inhibiting the biological functions, by blocking different functional groups of enzymes, polynucleotides and macromolecules (Kvesitadze et al. 2006). The toxic heavy metals and their sources are shown in Table 1 and the detrimental effects of metal ions on microbes, plants, and humans are shown in Table 2.

Table 1 Sources of heavy metal contamination

Heavy metal	Sources	References
Arsenic	Phosphatic fertilizers, metal-hardening, paints, textile, mining, pesticides, smelting	Raymond et al. (2011)
Cadmium	Phosphatic fertilizers, electronics, electroplating, chrome tanning, dyes, paper industries, pigments and paints	Malarkodi et al. (2007)
Chromium	Cement and waste ignition, chromium plating, coal and oil combustion, stainless steel welding, tanning, metal plating, tanning, rubber and photography	Joshi (2003)
Copper	Electroplating industries and electrical wastes	Joshi (2003)
Lead	Paints, battery, gasoline additives, cable covering, explosives, and antifouling paints	Li et al. (2015)
Nickel	Electroplating, iron steel, paint, powder, battery-processing units	Joshi (2003)
Mercury	Paints, pharmaceutical wastes, disinfectants, pulp and paper industries, scientific instruments, urban sewage treatment	Pai et al. (2000), Pirrone et al. (2001)

Table 2 Effects of heavy metals on microbes, plants, and humans

Heavy metals	Effect on human	Effects on plants	Effects on microbes	References
Arsenic	Skin cancer, cardiovascular and respiratory disorder, conjunctivitis brain damage, dermatitis	Creates physiological ailments, oxidative stress, inhibits root growth, destabilize cell membrane fluidity and fruit production	Enzyme inactivation and protein denaturation	WHO (2001), Finnegan and Chen (2012)
Cadmium	Causes bone and autoimmune diseases, cancer and red blood cell destructions, disorders in kidney and lungs	Affects plant growth, photosynthesis, enzyme activities and seed germination, causes chlorosis	Denatures protein and nucleic acid. Interferes the transcription factors	Bhattacharyya et al. (2008), Fashola et al. (2016), Sobha et al. (2007)
Chromium	Causes diarrhea, and many respiratory diseases like bronchopneumonia, chronic bronchitis, emphysema, allergic reactions like headache and skin itching	Physiological disorders like delayed senescence, wilting, chlorosis Biochemical lesions affect germination and cause stunted overall development	Elongates the lag phase of cell division, affects growth, and reduces oxygen uptake	Cervantes et al. (2001), Barakat (2011)
Copper	Causes anemia, diarrhea abdominal pain, headache, liver and kidney damage, interacts with prion proteins	Causes stunted and retarded growth. Causes chlorosis and oxidative stress	Disrupts many cellular functions and inhibits many enzyme activities	Khan et al. (2008), Duruibe et al. (2007), Dixit et al. (2015), Fashola et al. (2016)
Lead	Diseases like Alzheimer's, insomnia, anorexia, nephritis, infertility, renal system damage, neuron damage Increases blood pressure	Affects CO_2 fixation and growth. Inhibits seed germination and enzyme activation	Denatures protein and nucleic acid. Interacts with transcription factors	Mupa (2013), Fashola et al. (2016)
Nickel	Causes cardiovascular diseases and other symptoms like dry cough, dermatitis, chest pain, dizziness, lung and nasal cancer, breathing difficulties, headache, kidney diseases	Reduces nutrient uptake and chlorophyll content. Causes protein denaturation	Disturbs cell membrane fluidity and causes protein denaturation	Malik (2004), Fashola et al. (2016), Rascio and Izzo (2011)

(continued)

Table 2 (continued)

Heavy metals	Effect on human	Effects on plants	Effects on microbes	References
Mercury	Causes blindness, deafness, dementia, dizziness, and affects fertility rates, gastrointestinal irritation, and gingivitis	Affects oxidative system and plant growth. Induces genotoxic effect	Affects diversity and population size. Denatures and disrupts proteins and cell membranes	Fashola et al. (2016)

The environmental laws are very stringent on the release of effluents containing different heavy metals into soil and water bodies. It is mandatory to reduce the concentration of heavy metals to below permissible limits before release of effluents. Many physicochemical methods currently have been adopted to treat heavy metal pollution.

Chemical precipitation of heavy metal is done by the addition of flocculation agents such as $FeCl_2$ or $FeCl_3$, $Al(OH)_3$, $CaCO_3$, and other organic polymers under controlled pH (Aziz et al. 2008). This method is very simple with low capital cost. However, the generation and disposal of sludge and high operational costs are the main challenges with this method (Fu and Wang 2011). In another method called adsorption, heavy metals are separated from liquid system using a suitable adsorbing agent. This method is purely based on physicochemical interaction of sorbate and metal ions. Waste biomass from agriculture, by-products of different industries, and modified biopolymers are mainly used as low-cost adsorbents. This method works well in a wide range of pH, has higher metal binding capacities, and requires low operating cost. But low selectivity and generation of waste products make this process non-attractive (Gupta et al. 2001; Kurniawan et al. 2006). Filtration is another physical process widely applied to separate heavy metals with the aid of a semipermeable membrane of various molecular sizes. Several kinds of membrane filtration processes such as ultrafiltration, reverse osmosis, and nano-filtration are adopted to eliminate heavy metals from wastewater. Apart from heavy metals, membrane filtration is also used for removing suspended solids and organic compounds. This method requires small space and low pressure and has high selectivity (Kurniawan et al. 2006), but this method is limited by high input material cost and short membrane life. Electrodialysis is another approach where specific heavy metal is separated through semipermeable or ion-selective membranes (Mohammadi et al. 2004). But it is limited by the slow and high input material cost. The conventional physicochemical methods are analyzed as costlier, nonrenewable, and non-eco-friendly approaches. Hence, the need of alternative, cheap, efficient, and environmentally friendly biological approaches are essential (Hoang et al. 2018) for removal of heavy metals and bioremediation by bacteria and algae is one such approach.

2 Bioremediation

Bioremediation is a process that deploys plants, microbes, or their enzymes to treat contaminated environment. Heavy metal bioremediation encompasses elimination of heavy metals from soil and sewage water by using living or dead organisms. Microbes such as bacteria, algae, and their consortia have proven to be potential for heavy metal removal from wastewaters. The microbial agents used in bioremediation process should be safe to handle, easy to cultivate and apply, tolerant to heavy metals, renewable, and easy to separate after treatment. Bioremediation is easy to perform, cost-effective, helps in metal recovery, efficient at very low metal concentrations, and does not generate sludge or secondary pollution (Ilamaran et al. 2018). Application of biological systems for removal of metal ions from contaminated water reduces the cost of bioremediation and acts as an alternative approach to current physicochemical remediation technologies.

Bioremediation by living organisms mainly depends on microbial growth and the effectiveness of bioremoval depends on optimal physical factors like pH, temperature, and nutrients (Li et al. 2017). The pH levels below 3.0 and above 9.0 affect metal solubility, bioavailability, and microbial growth (Mani and Kumar 2014). The pH level between 5.0 and 9.0 is the most optimum to various microorganisms and maximum bioremediation occurs (Enim 2013). The most optimum temperature for maximum microbial growth is between 20 and 40 °C and at this optimum temperature metal solubility is good and doubling time of the microorganism is relatively short. The growth phase of a microbe is also a very important parameter in bioremediation. At log phase the microbe is very active; produces maximum enzyme and biomass. Thus maximum bioremediation happens in this phase (Gikas et al. 2009).

The isolated and cultured bacteria or algae are generally tolerant to one or two heavy metals. However, they might be sensitive to other heavy metals too. The industrial effluents may contain >1 metal with varied concentrations and may be loaded with inorganic and organic compounds. These would affect the growth and efficacy of bioremediation. Hence there is a need for efficient and robust biological system comprising different groups of microbes. The consortia of algae and bacteria is one such approach, where in nature these two coexist, mutually get benefitted, and can withstand the changing environmental conditions. In this chapter review reports of previous researchers on the source of heavy metal, toxic effects, bioremediation mechanisms, and metal-removing capacities of bacteria, algae, and algae-bacterial consortia are discussed. It also discusses the attributes of bacteria in promoting algal growth for effective bioremediation. Not only findings, the analyses on consortium of bacteria and algae are summarized here to suggest future directions.

3 Mechanisms of Bioremediation

Metal resistance properties of microorganisms are believed to be evolved over the years because of their exposure to toxic metals (Hrynkiewicz and Baum 2014). Some mechanisms are specific and are plasmid borne but other mechanisms are general and confer resistance to a variety of metals (Zhang et al. 2018). The bioremediation mechanisms are complex, and difficult to understand. Algae and bacteria exhibit and share many similar mechanisms of heavy metal removal (Fig. 1). These mechanisms could be either metabolism dependent or independent (Ayangbenro and Babalola 2017) and each mechanism is briefly discussed here with specific examples.

3.1 Biosorption

Biosorption is a metabolism-independent process. Occurrence of several functional groups on cell wall such as amine, carboxyl, hydroxyl, and phosphate groups is involved in metal biosorption, through the ionic and physical interaction (Fomina and Gadd 2014). The gram-negative bacterial cell walls is mostly composed of lipopolysaccharides (LPS), phospholipids, and proteins (Escudero et al. 2018). Gram-positive microbes have more amino acids than lipids whereas gram-negative bacteria have more lipids than amino groups (Turner et al. 2018). Gram-positive and gram-negative bacterial population remarkably differ in their biosorption capacities. Gram-positive bacteria show less biosorption, due to heavily cross-linked peptidoglycan content and less surface complexation. Exchange of metal ions occurs with counterions found on different functional groups of cell walls (Shen et al. 2008).

The cell wall composition of algae makes them the best biosorbent agents. The brown algal cell wall is composed of cellulose, alginic acid, and sulfated polysaccharides (Deniaud-Bout et al. 2017). The cell wall of red algae is made up of cellulose and sulfated polysaccharides. Whereas the green algal cell wall is majorly made up of cellulose and glycoproteins. Presence of amino, carboxyl, sulfate, and hydroxyl group on the cell wall imparts negative charges to the cells which are mainly involved in metal ionic interaction. The presence of alginates (comprises with 1500 units of β-ᴅ-mannuronic acid and 1, 4-linked α-ʟ guluronic acid) and fucoidan (sulfate or acetate molecules and 1–3-linked α-ʟ-fucopyranose) in brown algae provides additional electronegativity for heavy metal biosorption (Deniaud-Bout et al. 2017; Brawley et al. 2017).

Biosorption is the major mechanism of bioremoval, observed found in bacteria and algae. The mechanism of metal removal by biosorption is comparatively faster than accumulation and transformation (Anastopoulos and Kyzas 2015). However, biosorption process is mainly affected by many extrinsic parameters. Factors influencing effective biosorption are briefly discussed here.

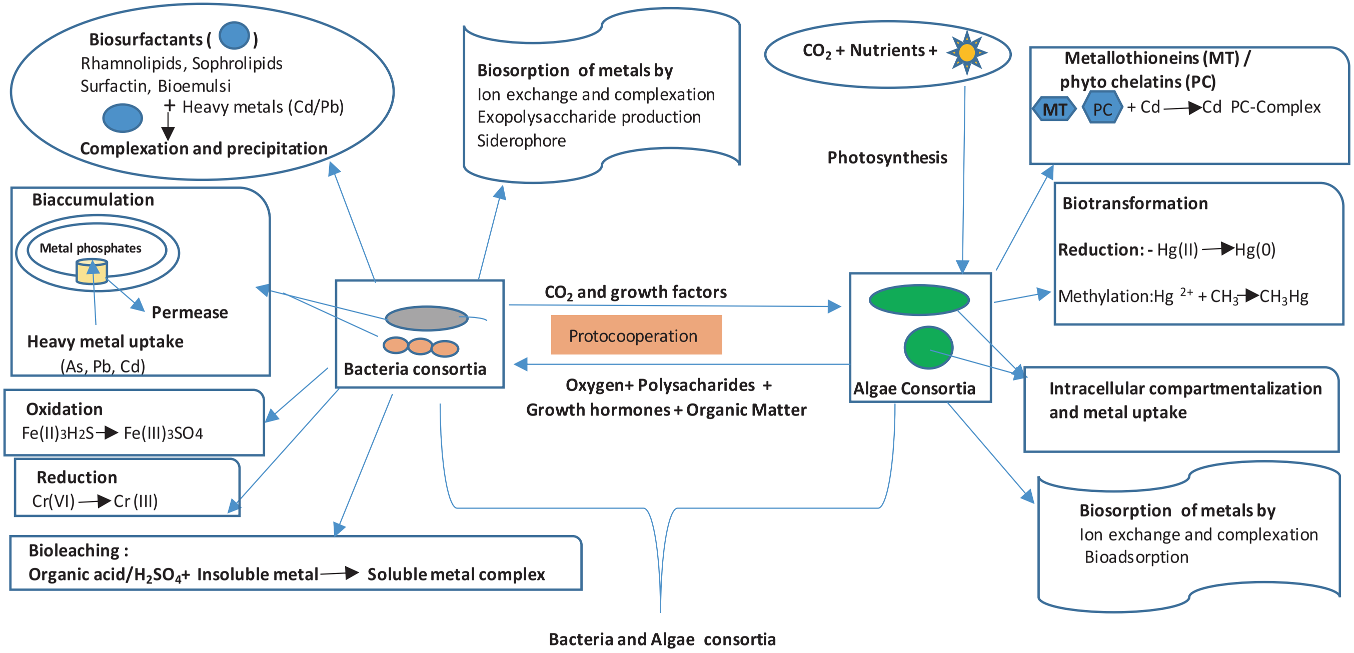

Fig. 1 Bacteria and algae protocooperation and heavy metal remediation. The heterotrophic bacteria produce CO_2, which is utilized by autotrophic algae. The O_2 produced during photosynthesis of algae would be utilized by bacteria. The biosorption of metal depends on cell wall composition and it is the first step in bioaccumulation. The membrane proteins (permease) involve in transfer of metals from outside to inside the cell, where it is complexed with protein molecules and gets stored. The EPS and siderophores secreted by bacteria and algae are involved in metal complexation and bioremoval. The biotransformation or oxidation reduction of heavy metals depends on the enzyme activity in which the metals are converted from very high toxic form to less toxic form or insoluble form to soluble form

3.1.1 Effect of pH

The pH of media affects metal solubility and total charge on biosorbent. Biosorption increases with increasing pH to a certain extent and vice versa. The free hydrogen ions at lower pH reduce the surface adsorption of heavy metals by increasing overall positive charges on functional groups of adsorbents. At higher pH, due to the protonation and lower number of H^+ ions, functional groups with negative charges start attracting positively charged metal ions and increase the biosorption rate (Feng et al. 2011).

3.1.2 Primary Concentration of Metal Ion

The initial concentration of metal ion is a key driving force which affects the biosorption rate and mass transfer resistance between solid and aqueous phases. The biosorption rate increases with rise in preliminary metal ion concentration until complete saturation of binding sites on the biosorbents (Pahlavanzadeh et al. 2010). The 100% adsorption happens at lower concentration of metal ions due to freely available functional groups whereas at higher concentration due to saturation of binding sites, many ions are left unadsorbed (Naiya et al. 2009). Chatterjee et al. (2010) observed the increase in biosorption rate from 25 to 175 mg L^{-1} with a maximum of 175 mg L^{-1} for different metal ions Fe^{3+}, Cr^{3+}, Co^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Ag^{2+}, and Pb^{2+} using a dead biomass of *Geobacillus thermodenitrificans*.

3.1.3 Effect of Biomass Dosage

Concentration of biomass is one of the prime factors which affect biosorption rate. Biomass provides functional groups or active sites for metal sorption (Chong et al. 2013). Increase in biomass concentration provides greater surface area and more functional groups (Kumar and Gaur 2011). Abdel-Aty et al. (2013) described the consequence of biomass dosage on biosorption of Cd^{2+} and Pb^{2+} metal ions using algal biomass *Anabaena sphaerica*. It was reported that enhancement in biosorption with increasing biosorbent dose, which become constant at higher concentration. This might be due to the formation of aggregation at higher biomass concentration, resulting in reduced surface area for biosorption.

3.1.4 Effect of Contact Time

The optimum contact time is the time required for enough interaction of all the metal ions present in the solution with the biomass functional groups. The biosorption increases with increasing contact time and decreases after saturation. The maximum biosorption at optimum biomass concentration with minimum contact time determines the cost of biosorption process (Abdel-Ghani et al. 2007).

3.2 Intracellular Complexation

Intracellular sequestration is achieved by complexation of metal ions by proteins or several compounds inside the cells. Biosorption is the first mechanism used for concentrating the metal ions on cell surface followed by slow transport for metal complexation. The live cells transport heavy metals across cell membrane, and transform, detoxify, accumulate heavy metals (Malik 2004). The bioaccumulation is affected by many external factors such as cell age, growth, media composition, contact time, pH, temperature, and metal and biomass concentration (Wang and Chen 2006).

Metallothioneins (MT) are cysteine-rich, metal-binding low-molecular-weight proteins, known to lower the free metal ion concentration within the cell cytoplasm (Blindauer 2011). MT are produced or induced in response to the occurrence of heavy metals in surrounding environment, have high affinity for various metal ions, and were observed in *Cyanobacterium*, *Synechococcus* sp., as well as *E. coli* and *Pseudomonas putida* (Andrews 2000). There are three classes of MT. Class I MT are polypeptides/proteins observed in vertebrates and fungi (*Agaricus bisporus* and *Neurospora* sp.) and are not reported in algae. Class II MT are specific proteins/polypeptides present in algae (cyanobacteria) and higher plants. Class III MT are also called as phytochelatins and are non-translationally synthesized short-chain polypeptides found in certain algae, fungi, and higher plants (Mejare and Bulow 2001). MT are synthesized in the cytosol and exported to the chloroplast and mitochondria. This phenomenon was first reported in *Euglena*, where nearly 60% of the accumulated Cd^{2+} were found in the form of Cd-MT III complexes in chloroplast (Soldo et al. 2005). In case of *P. putida* low-molecular-weight cysteine-rich proteins were known to involve in copper, cadmium, and zinc sequestration (Higham et al. 1986). Glutathione was known to involve in Cd^{2+} intracellular sequestration in *Rhizobium leguminosarum* (Lima et al. 2006).

3.3 Extracellular Sequestration

This is the mechanism of metal ion removal by complexation and precipitation as insoluble compounds in the periplasmic space or outside the cell wall. The CopA and CopB (periplasmic) and CopC (outer membrane) copper-inducible proteins were known to secrete polymers and sequest copper by *Pseudomonas syringae* (Cha and Cooksey 1991). There are many efflux or transport systems identified in bacteria which are known to transport metals from inside cytoplasm to outside. In case of *Synechocystis* PCC 6803 strain, zinc is transported from cytoplasm to periplasm by efflux system (Thelwell et al. 1998). Various efflux pumps have been identified in many archaeal and bacterial genera. The efflux pumps such as *PbrA* from *Cupriavidus metallidurans* strain CH34, *ZntA* from *E. coli*, *CadA* from *S. aureus*, and *CadA2* from *Pseudomonas putida* KT2440 are recognized to transport Pb^{2+} as well as Cd^{2+} and Zn^{2+} to the periplasm from cytoplasm (Leedjarv et al. 2006; Hynninen and Virta 2010). *P. aeruginosa* (Wang et al. 2002) and *Vibrio harveyi* (Mire et al. 2004) strains precipitate lead as sulfide and phosphate salts, respectively, in the periplasmic space. Under anaerobic conditions *Klebsiella planticola* produces hydrogen sulfide from thiosulfate and precipitates Cd^{2+} as insoluble sulfides (Sharma et al. 2000).

Siderophores are low-molecular-weight organic compounds which can chelate metals (Banik et al. 2016). The main biological role of siderophore is to quench and transport iron into the cell. Hydroxamate and catecholate siderophores are the two major groups of siderophores. Hydroxamate groups strongly bind ferric iron (Amin et al. 2009). Certain heavy metal ions such as gallium, aluminum, and chromium are of similar size to ferric iron; make complexes with hydroxamate groups; and reduce metal toxicity. Siderophores produced by *Anabaena* sp. make complexation with copper ions and reduce toxicity (Rudolf et al. 2016). The presence of metal ions other than iron is also known to stimulate siderophore formation in several bacteria. In *Bacillus megaterium* the presence of aluminum, copper, chromium, cadmium, and zinc increases siderophore production. The induction of siderophores in response to heavy metal is sometimes beneficial which decreases the metal toxicity (Hu and Boyer 1996).

Exopolysaccharide (EPS) is a high-molecular-weight secretory product of microbes, and protects cells from adverse environmental conditions, phagocytosis, and parasitism. The secretion of EPS is sometimes induced or it is an inherent property of a cell. These are composed of polysaccharides, carbohydrates, fatty acids, and sometimes nucleic acids (Lau et al. 2005). The binding of EPS to metal ion is due to the occurrence of negatively charged functional groups such as succinyl, hydroxyl, phosphate, and uronic acids. The mobilization of metal ions offers protection to cell from the deleterious effects of metal ions (Zhang et al. 2018).

3.4 Reduction of Heavy Metals

In this process microbes convert metals from toxic to nontoxic forms by changing their oxidation status by oxidoreduction process. Metals serve as electron donors or acceptors, under anaerobic condition. The oxidized form of metal serves as terminal acceptors for electrons and energy generation. The enzymes secreted by microbes involve in reduction of metals from one form to another (Barkay et al. 2003; Viti et al. 2003). *Geobacter metallireducens* is a strict anaerobe that reduces Mn (IV) to Mn (II), and U(VI) to U(IV). *G. sulfurreducens* and *G. metallireducens* can decrease chromium (Cr) from the very lethal Cr (VI) to less toxic Cr (III) (Bruschi and Florence 2006).

3.5 Methylation of Metals

Methylation is a metal transformation process, where methyl groups form bonds with heavy metal ions and alter solubility and volatility (Thayer 2002). Methylation of metals facilitates mobilization of metal ions and diffuses toxic elements away from cells. Volatilization of metals has been observed with metals such as Pb, Hg, Sn, and As (Clemens and Ma 2016). In volatilization of Hg^{2+}, the metal is readily oxidized to dimethyl mercury and methylmercury which diffuse out readily from cells (Gadd 2004). Many bacteria, including *Aerobacter aerogenes, E. coli, Clostridium, Pseudomonas, Bacillus,* and *Mycobacterium* can methylate mercury. Most of the members of sulfate-reducing bacteria (SRB) play a key role in the methylation of metals (Gomathy and Sabarinatha 2010).

4 Bacterial Bioremediation of Heavy Metals

Bacteria can thrive well in all adverse conditions and have wide metabolic activities, higher surface-to-volume ratio, and higher multiplication rates (Ayangbenro and Babalola 2017). Bacteria remove heavy metals by biotransformation, or biosorption. Biotransformation of heavy metal is dependent on the metabolic activity of bacteria. Bacterial enzymatic redox reactions convert soluble heavy metals into insoluble forms (Silvia et al. 2013). The rate of these reactions is influenced by direct concentrations of microbial biomass and enzymatic activity (McLean and Beveridge 2001). Cr^{6+} reduction using an isolated bacterium from industrial effluents was studied (Ran et al. 2016). The isolated bacterium *Sporosarcina saromensis* M52 was able to tolerate 500 mg Cr^{6+} L^{-1} and was used for degradation of chromium. The physical parameters were optimized to remove maximum chromium by reduction mechanism. It was found that 50–200 mg Cr^{+6} L^{-1} was removed in 24 h at pH 7.0–8.5 and 35 °C. The complete degradation of 100 mg Cr^{+6} L^{-1} was observed at pH 8.0 and 35 °C in 24 h. The biomass of dead and living bacteria acts as an excellent biosorbent. The removal of metals using bacterial biomass is an economical and renewable approach. Modification of functional groups of bacterial cell wall by many chemicals is known to enhance the biosorption process (Ghaedi et al. 2013). The nickel (Ni^{2+}) and cobalt (Co^{2+}) metal ion uptake by purple photosynthetic bacterium *Rhodobacter sphaeroides* strain R26 was investigated by Caltaliano et al. (2009). Here they studied the metal ion interactions, interms of passive and active uptake. The maximum uptake of cobalt and nickel were found to be 2.2 and 0.5 mg g^{-1} of dried biomass of *Rhodobacter sphaeroides*, respectively. The carboxylase groups found on gram-negative bacterial cell wall were playing a major role in surface binding of heavy metals; in this study most of the metals were accumulated on the cell surface.

The involvement of EPS in lead immobilization was observed in *Staphylococcus aureus*, *Micrococcus luteus*, and *Azotobacter* sp. (Maier et al. 2009). *Stenotrophomonas maltophilia* EPS showed 43% and 69.1% removal of chromium and copper ions from initial concentration of 25 mg L^{-1}, respectively (Kiliç et al. 2015). *Methylobacterium organophilum* EPS was found to remove 18% Pb^{2+} and 21% Cu^{2+} from 0.04 ppm initial metal concentration (Kim et al. 1996). *Enterobacter cloacae* EPS was found to remove 20% Cd^{2+}, 20% Cu^{2+}, and 75% Cr^{6+} from 100 mg L^{-1} initial metal concentration (Iyer et al. 2005; Gutierrez et al. 2012).

Biosurfactants are compounds excreted by many microbes as they can form complex and increase solubility of metals, thereby reduce metal toxicity. Many researchers found that the diversity of biosurfactant-producing bacteria is more in metal-contaminated environment than in uncontaminated ones (Tang et al. 2018). Abdurrahim et al. (2012) investigated the feasibility of using rhamnolipid, a biosurfactant produced by *Pseudomonas* sp., to remove or reduce heavy metals (Cd^{2+}, Pb^{2+}, Ni^{2+}, Ba, Zn^{2+}, and Sr). Different concentrations (20, 40, and 80 ppm) of rhamnolipids were evaluated for metal removal efficiency. The results showed that 80 ppm of rhamnolipids reduced up to 53%, 62%, 56%, 28%, 20%, and 7% of Cd^{2+}, Pb^{2+}, Ni^{2+}, Ba, Zn, and Sr, respectively.

Ramaiah et al. (2008) studied the cadmium removal capacity of *P. aeruginosa* and *A. faecalis*. *P. aeruginosa* was found to remove 70% cadmium from an initial concentration of 1000 mg L^{-1} to 17.4 mg L^{-1}. Whereas *A. faecalis* removed 75% cadmium from 1000 mg L^{-1} to 19.2 mg L^{-1} in 72 h of incubation time. 78% bioremoval of chromium was also reported from an initial metal concentration of 16 mg L^{-1} using *Acinetobacter* sp. and *Arthrobacter* sp. consortium. Ashruta et al. (2014) reported 75–85% removal of chromium, zinc, cadmium, lead, copper, and cobalt using a consortia of bacteria in less than 2-h time. Singh et al. (2013) reported 72% Cr (VI) removal from an initial metal concentration of 1000 μg/mL using facultative microbe *B. cereus*. The removal was observed under a wide range of temperature (25–35 °C) and pH (8–10). However, the maximum was observed at optimum temperature of 37 °C and pH 8.0. In the field study more stable and better surveillance of bacteria was observed in mixed culture rather than in single isolate (Sannasi et al. 2006). The consortia of right bacteria were found to be metabolically superior and were more appropriate for field applications (Kader et al. 2007). Ramaiah et al. (2008) and Kim et al. (2015) immobilized *Desulfovibrio desulfuricans* on zeolite and observed 99.8%, 98.2%, and 90.1% removal of chromium (Cr^{6+}), copper (Cu), and nickel (Ni), respectively, in a batch process. The different types of bacteria used for removal of various kinds of heavy metals are mentioned in Table 3.

5 Algal Bioremediation of Heavy Metals

The algae are photosynthetic organisms and able to grow autotrophically, mixotrophically, and heterotrophically. Algae fix atmospheric carbon dioxide (CO_2) and generate oxygen (O_2) using sunlight. The microalgal bioremediation has emerged as a low-cost alternative to conventional methods (Zeraatkar et al. 2016). Algal biotechnology is gaining popularity with the need for novel renewable eco-friendly technologies and the expansion of state-of-the-art mass production of algae for

Table 3 List of bacteria and algae used for different heavy metal removal according to some published references

Group of microorganisms	Bacteria	Metal	References
Bacteria	*Bacillus laterosporus* *Bacillus licheniformis* *Alcaligenes* sp., *Pseudomonas* sp. *Moraxella* sp. *Pseudomonas veronii* *Enterobacter* sp. J1	Cd^{2+}	Springael et al. (1993), Zouboulis et al. (2004), Quintelas et al. (2009)
	Enterobacter cloacae	Pb^{2+}	Kang et al. (2015)
	Bacillus subtilis *Pseudomonas licheniformis*	Ni^{2+}	Holan et al. (1994)
	Pseudomonas putida *Enterobacter cloacae* B2-DHA *Bacillus subtilis* *Bacillus cereus*	Cr^{6+}	Dong et al. (2013), Balamurugan et al. (2014), Ramanan et al. (2016)
	Bacillus licheniformis	Cu^{2+}	Beveridge (1989)
Algae	*Oscillatoria quadripunctulata* *Oscillatoria tenuis* *Scenedesmus acutus* *Spirogyra* sp. and *Cladophora* sp.	Pb^{2+} Pb^{2+}	Azizi et al. (2012), Rana et al. (2013)
	Scenedesmus acutus *Scenedesmus quadricauda* *Chlorella vulgaris* *Spirogyra hyaline*	Cd^{2+}	Matsunaga et al. (1999), Cannizares et al. (2001), Kumar and Oommen (2012), Shanab et al. (2012)
	Spirogyra sp. and *Spirulina* sp.	Cr^{6+}	Mane and Bhosle (2012), Coelho et al. (2015)

biofuel production, carbon mitigation, and bioremediation. The algal biomass is a rich source of carbohydrates, lipids, and proteins that can be used in producing biofuels or other value-added products. The structure of microalgae, faster growth, bigger cell size, extracellular and intracellular mechanisms, their capability to proliferate in the presence of higher concentration of heavy metals, and widespread occurrence have made them a suitable candidate for bioremediation (Mitra et al. 2012).

The different functional groups present on algal cell wall determine the biosorption. The biosorption of three algal groups is observed in the following orders of Chlorophyta < Phaeophyta < Rhodophyta (Al-Shwafi and Rushdi 2008). Many algae were studied and applied for biosorption of heavy metals. *Oedogonium rivulare* and *Cladophora glomerata* were most widely used algal species for continuous removal of Cd, Co, Cr, Cu, Fe, Mn, Ni, and Pb from contaminated water. The dried algal biomass of *Caulerpa lentillifera* is an excellent remover of multiple metal ions. The *Spirogyra* is known to remove 58–85% of Cu^{2+} from 20 mg L^{-1} initial concentration in 30 minutes (Pavasant et al. 2006).

The batch-mode biosorption of copper (Cu) and zinc (Zn) with isolated *Chlorella* sp. was studied under optimum conditions in a Na-alginate immobilized system. In an immobilization mode *Chlorella* sp. biosorbed Cu and Zn at rates of 33.4 and 28.5 mg g^{-1} of biomass, respectively. The biosorption in immobilized mode was found to be higher than the non-cell immobilization mode. Scanning electron microscopic study and X-ray spectroscopic study revealed that Cu and Zn were mainly attached to external cell surface in the biosorption. Naoto et al. (2006) isolated heterotrophic algae resistant to Cd^{2+} (4 mM) and identified as *Chlorella sorokiniana*. This heterotrophic alga was also known to tolerate and bioadsorb, Cd^{2+}, Cu^{2+}, and Zn^{2+} at 43.0, 46.4, and 42.0 μg mg^{-1} dry weight, respectively.

The individual and combined copper (Cu) and cadmium (Cd) metal biosorption study was conducted using *Scenedesmus abundans* (Patricia and Stone 2002). In the study dead and live cells were used; the living cells showed more biosorption than dead cells. The developed biosorption curve for the metal removed using live cells of *S. abundans* shows that the biosorption was decreased with an increasing biomass concentration. Competitive effects between the metals (Cu and Cd) were observed at lowest biomass concentration and no competition was observed at higher concentration of metals studied (1–7 mg L^{-1}).

The study with different freshwater algal cultures *Cladophora glomerata*, *Vaucheria debaryana*, *Oedogonium westie*, and *Zygnema insigne* for removal of Cd, Cr, and Pb has shown that *C. glomerata* was able to remove all the metal species used in the study through biosorption and bioaccumulation (Cr, Cd, and Pb). The metal removal by *C. glomerata* was in the order of Cd > Cr > Pb (Isha et al. 2015). The bioaccumulation capacity of algae made them to be used as biomonitors of metal ions in marine systems (Gosavi et al. 2004).

Algae can survive both in fresh and seawater (Anastopoulos and Kyzas 2015) and are known to effectively remove and bioaccumulate metal ions from aqueous solution (Mehta and Gaur 2005). The bioaccumulation especially occurs in intracellular spaces or cell vacuoles (Chen et al. 2012). The bioremoval capacity of algae varies with type and species, and dead or live algae. Dead algal biomass is known to remove more metal than live algae (Mehta and Gaur 2005).

Goher et al. (2016) studied the removal of cadmium, copper, and lead using dead biomass of *C. vulgaris* and they have observed 95.5%, 97.7%, and 99.4% removal, respectively, from mixed solution of 50 mg L^{-1} of each metal ion. Perez-Rama et al. (2002) studied the biosorption of cadmium using live cells of marine microalga *Tetraselmis suecica.* In the study Cd (II) removal was measured as total removal (surface accumulation and intracellular accumulation) after 24 h and 72 h of exposure to different concentrations of Cd (II) (0.6–45 L^{-1}). The biosorption of 40.22 $mgL^{-1}g^{-1}$ of biomass was observed after 72 h and at lower concentration the metal was intracellularly accumulated and removed. Abdel-Aty et al. (2013) studied the biosorption of Cd (II) and Pb (II) using a blue green alga *Anabaena sphaerica*. The maximum biosorption capacities were found to be 111.1 and 121.95 mg/g of biomass for Cd (II) and Pb (II), respectively. Kizilkaya (2013) evaluated the biosorption capacity of nonliving *Rivularia bulata* algal biomass and observed 26.36

and 34.3 mg/g of Cd (II) and Pb (II) removal, respectively. The different types of algae used for removal of various kinds of heavy metals are mentioned in Table 3.

6 Bacterial and Algal Consortium for Efficient Bioremoval of Heavy Metals

Bacteria are very small in size (1–2 μM). The small size contributes to higher surface area and they have higher growth rates and short doubling time. Bacteria are metabolically diverse, thrive well in all conditions of environment, and have wide metal tolerance (Ayangbenro and Babalola 2017). Likewise, algae are also a potent microbe, mainly found in nature as autotrophs, and possess many similar metal removal mechanisms like bacteria (Buchan et al. 2014). The industrial effluents contain a variety of heavy metals and other pollutants. The bioremediation of these wastes could be difficult to treat by a single microbe which has resistance to a single heavy metal. This can be addressed by a mixture of compatible microbes which have resistance to a variety of heavy metals and other pollutants (Loutseti et al. 2009). The bioremediation process involving consortia of right microbes can be an efficient system in reducing the pollution caused by many organic and inorganic wastes and heavy metals (Pires et al. 2013). The beneficial effects of algae bacterial symbiosis or consortium approach of algae and bacteria in removing heavy metal are discussed in detail. The consortia approach is illustrated in Fig. 2.

6.1 Alga-Associated Bacterial Diversity Analysis

The advancement in DNA sequencing methods helps in the identification of specific bacteria associated with algae. The microbiota analysis using next-generation sequencing (NGS) has led to identify alga-associated bacteria and it was observed that bacteria vary with the type of cultivation system and algae (Garcıa et al. 2017; Sun et al. 2018; Yang et al. 2018). *Nannochloropsis salina* cultivated in raceway pond was associated with Gammaproteobacteria and Alphaproteobacteria whereas photobioreactor-grown culture was associated with Deltaproteobacteria and Bacteroidetes. *Botryococcus braunii* cultured in flask was associated with Gammaproteobacteria, Betaproteobacteria, and Firmicutes (Carney et al. 2016; Sambles et al. 2017; Fulbright et al. 2018). The bacterial diversity analysis in the algal biofilm composed of *Chlorella vulgaris* and *Scenedesmus obliquus* was majorly composed of Alphaproteobacteria, Bacteroidetes, Betaproteobacteria, and Gammaproteobacteria; these four groups of bacteria constitute nearly three-quarters of the bacterial community in the biofilm (Krohn-Molt et al. 2013). The NGS-based bacterial diversity revealed that cultured microalgae were mostly associated with Proteobacteria; Gammaproteobacteria belongs to Cytophagales and Flavobacteriales orders.

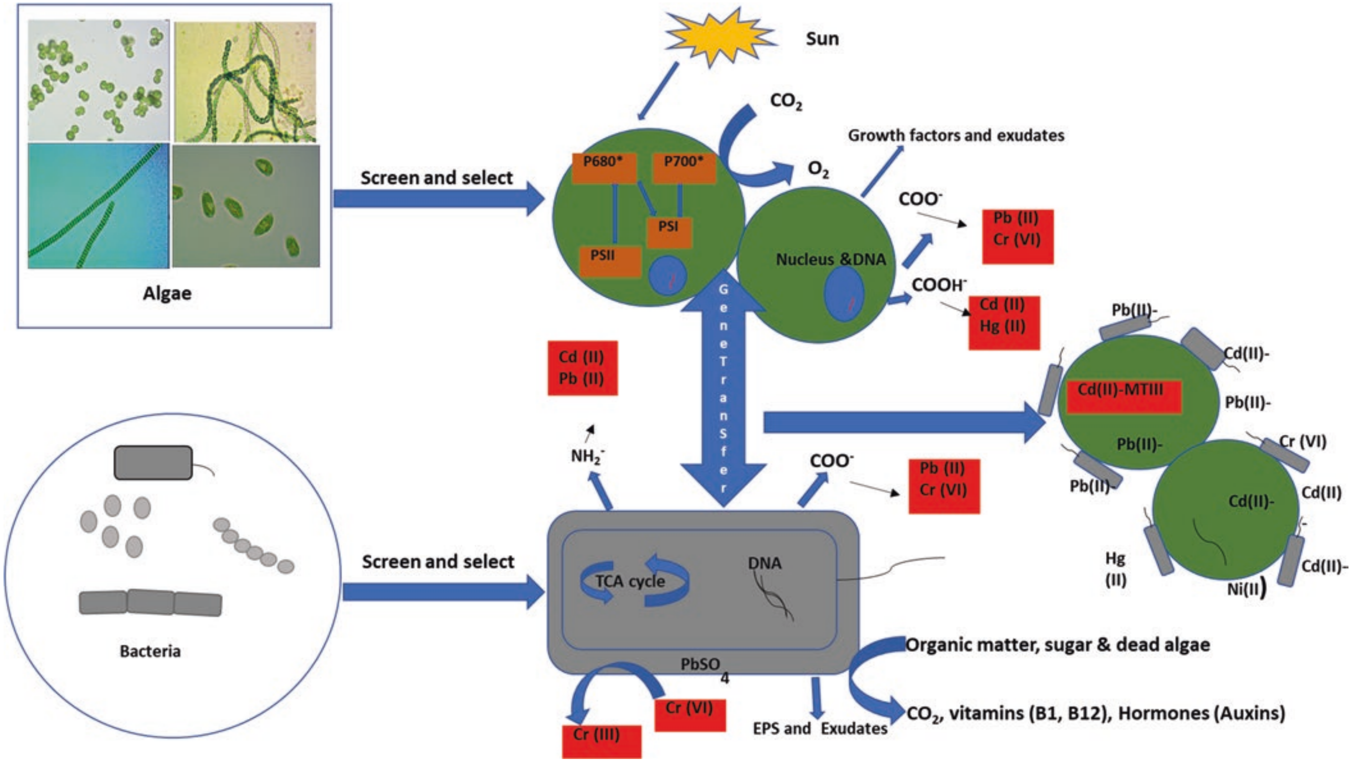

Fig. 2 Algae-bacteria consortia approach for heavy metal remediation. The efficient algae and bacteria which have highest tolerance for various metal ions will be selected for bioremediation of industrial effluents containing various heavy metals. The heterotrophic bacteria in the consortia metabolize organic load present in the effluents and produces growth-promoting substances for algae (e.g., vitamin B1, B12, growth hormones (auxins), CO_2, and fixed N_2) apart from bioremediation of heavy metals. Algae grow autotrophically by utilizing sunlight and dissolved CO_2 and produce O_2 required for bacterial growth and bioremoval of metals. In the association not only organisms get benefitted by mutual metabolite sharing but also horizontal gene transfer could happen between the organisms which impart robustness to changing environmental conditions. The algae and bacteria establish self-oxygenated robust system for heavy metal remediation

6.2 Roles of Bacteria in Promoting Algal Growth

In nature most of the algae live in symbiotic association with bacteria or fungi throughout their life cycle (Dittami et al. 2014; Amin et al. 2015) and are known to release signaling molecule to attract specific bacterial communities (Amin et al. 2012). These bacteria synthesize micronutrients, siderophores, growth stimulants, and antibiotics, which help in algal growth stimulation, spore germination, and pathogen resistance and to cope with the changing environmental conditions (Amin et al. 2009, 2012, 2015; Ramanan et al. 2016; Bruhn et al. 2007; Seyedsayamdost et al. 2011; Wahl et al. 2012; Natrah et al. 2013; Danchin and Braham 2017; Xie et al. 2013; Dittami et al. 2016). The associated bacteria, apart from enhancing algal growth, also help in faster degradation of organic and inorganic wastes and hazardous substances (Su et al. 2012; Luo et al. 2014; Cavaliere et al. 2017). There are evidences that algae such as diatoms *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* have acquired hundreds of genes predicted to be involved in cell wall assembly, DNA recombination, assimilation of nitrogen and carbon, and ornithine-urea cycle, from symbiotic association with bacteria during more than 200 million years (Bowler et al. 2008). Symbiotic associations may impart robustness to fluctuating environmental conditions and stabilization to associated members apart from mutual metabolite sharing (Borde et al. 2003).

Bacteria live in association with algae; however the role of most of the bacteria is not clear. Recent studies and research have demonstrated that mutualistic interactions of algae-bacteria are more predominant (Seymour et al. 2017). Many attempts were made to coculture bacteria with algae to evaluate the effects of bacteria on microalgal growth (Le Chevanton et al. 2013; Sison-Mangus et al. 2014; Biondi et al. 2017). *C. vulgaris* was cultivated by adding many groups of bacteria. All these bacteria were known to fix atmospheric nitrogen and promoted the growth of *Chlorella* (Hernandez et al. 2009). Addition of *Bacillus pumilus* had increased algal density by 150% in nitrogen-free media (Hernandez et al. 2009). The addition of four groups of bacteria such as *Rhizobium* sp., *Flavobacterium* sp., *Sphingomonas* sp., and *Hyphomonas* sp. into *C. vulgaris* was known to increase algal cell density by more than 100%, growth rate from 0.22 to 0.47 day^{-1}, biomass from 1.3 to 3.31 g L^{-1}, and lipid content from 22.4% to 28% (Cho et al. 2014). The addition of *Rhizobium* sp. to *C. vulgaris* has shown to increase cell count by 72% and growth rate by 11% (Kim et al. 2014). *Brevundimonas* sp. was found to increase the cell density of *C. vulgaris* three times (Park et al. 2007). Like *Chlorella*, beneficial effects were also observed with other algal species. *Dunaliella* sp. SAG 19.3 cultivated with *Muricauda* sp. and *Alteromonas* sp. was found to enhance the algal biomass by 22% and 26%, respectively (Le Chevanton et al. 2013). *Lobomonas rostrata* cultivated with *Mesorhizobium loti* is known to produce vitamin B12 and support the absolute requirement of algae (Grant et al. 2014). The cell density of *Phaeodactylum tricornutum* was increased by 55% when it was grown in the presence of Alphaproteobacterium strain 29 (Bruckner et al. 2011).

6.3 Bacteria-Aided Acclamation of Algae to Fluctuating Conditions

The physical parameters like temperature, pH, nutrient, and salinity are most important factors that affect the growth and distribution of algae and bacteria (Ras et al. 2013; Olsenz 2011). Many algae have acquired the genes from bacteria through horizontal gene transfer that helps evolution and adoption of algae to different and adverse environmental conditions. *Picochlorum* sp. SENEW3 has a wide salt tolerance (0.35–10.8%) as compared to its other species (Wang et al. 2014). The genome was found to contain many functional genes, of which 24 genes were acquired from bacteria (Foflonker et al. 2015).

In a study with algae and bacteria, algae showed the obsoluete requirement of bacteria for its survival in the new environment. The transfer of brown macroalga *Ectocarpus* sp. strain 371 from seawater to freshwater medium affected the growth in the absence of associated bacteria and the growth was restored by constructing the seawater bacterial microbiota (Dittami et al. 2016). In another study with the *Chlamydomonas*, the inhibitory effect of high temperature was nullified with the added bacteria. *Chlamydomonas reinhardtii* requires an optimum temperature of 20–32 °C (Schroda 2004); the transfer of *C. reinhardtii* from 25 °C to 45 °C induced cell death and loss of chlorophyll. The death of algae was due to the effect of high temperature on cobalamin synthesis pathway and it was restored by adding extraneous cobalamin or by co-culturing alga with a cobalamin-producing *Sinorhizobium meliloti*. Hence, bacteria improved the robustness of algae and helped to adopt to the fluctuating environmental conditions.

6.4 Bacteria-Aided Nutrient Supplementation to Algal Growth

Algae need CO_2, inorganic nitrogen (N), phosphate (PO_4), and micronutrients (Singh and Das 2014) for growth. Media cost is significantly high for large-scale algal cultivation; lab-grade nutrients are most expensive; recycling or providing these nutrients via bacteria will make the process economically feasible (Clarens et al. 2010). The availability of inorganic carbon in the media/in natural water bodies is very less due to poor CO_2 gas transfer efficiency from atmosphere (Putt et al. 2011). The limitation of substrate availability can be overcome by additional supply of inorganic sodium bicarbonate or CO_2, but it adds additional cost to the cultivation (Clarens et al. 2010). The CO_2 released by bacterial degradation of organic compounds can act as an additional source of carbon for algae under carbon-limited conditions (Mouget et al. 1995; Subashchandrabose et al. 2011). This was demonstrated by addition of heterotrophic bacteria from domestic wastewater treatment into *Chlorella* sp.; this has increased the algal biomass by 3.4–4.8-fold (Bai et al. 2015). Nitrogen-fixing bacteria provide nitrogen to the algae (Singh and Das 2014);

Bacillus pumilus ES4 was found to enhance the growth of *Chlorella vulgaris* by fixing N_2 from atmosphere (Hernandez et al. 2009). The cyanobacterial algae *Richelia intracellularis* and *Calothrix rhizosoleniae* were found with diatoms in the oligotrophic ocean (Fiore et al. 2010). These diatoms showed higher growth rates in association with nitrogen-fixing cyanobacteria and during the association the nitrogen-fixing rates of cyanobacteria were increased by 171–420-fold as compared to free-living cyanobacteria (Foster et al. 2011). Algae can take up phosphorous in the form of inorganic phosphorous (Pi) Bacteria help in solubilization and mineralization of phosphorus through the secretion of phosphatases (Kononova and Nesmeyanova 2002). The dead algal cells act as a source of nitrogen, carbon, and phosphorus and can be recycled back for meeting the nutrients of algae or bacteria through bacterial application. This was demonstrated with *Gordonia* sp. txj1302RI and *Burkholderia* sp. txj1302Y4, where these two bacteria were involved in the transformation of dissolved organic phosphate (Po) to inorganic phosphate (Pi) and supported the growth of *Microcystis aeruginosa* (Zhao et al. 2012). Bacteria also supply the growth hormones (Amin et al. 2012, 2015; Sule and Belas 2013; Segev et al. 2016), vitamins (Croft et al. 2005; Grant et al. 2014), siderophores (Amin et al. 2009), and antibiotics (Seyedsayamdost et al. 2014). The heterotrophic *Dinoroseobacter shibae* DFL12T, in association with dinoflagellate host, provides B1 and B12. The analysis of the nutritional requirement of 326 algal species indicates the requirement of vitamin B12 and in most cases it was supplied externally or by its bacterial symbionts (Croft et al. 2005). The growth hormone, indole-3-acetic acid (IAA) secreted by *Sulfitobacter* sp. SA11 was known to promote cell division in algae. Siderophore produced by a *Marinobacter* sp. was known to promote iron assimilation in *Scrippsiella trochoidea* (Amin et al. 2009). The growth hormone phenylacetic acid and a broad-spectrum antibiotic tropodithietic acid produced by *Phaeobacter gallaeciensis* were known to control the pathogenic bacteria and promote the growth of *Emiliania huxleyi* algae.

6.5 Consortia of Algae and Bacteria as Bioremediating Agents

The bioremediation process involving consortia of right microbes can be an efficient system in reducing the pollution caused by many organic and inorganic wastes and heavy metals (Pires et al. 2013). This was addressed by a mixture of compatible microbes which were resistant to a variety of heavy metals and other pollutants (Loutseti et al. 2009). Pena et al. (2005) used the high-rate algal ponds (HRAP) and algal turf scrubber (ATS) holding biomass of algae (*Scenedesmus*, *Chlorella*, and *Cladophora*) and bacteria (cyanobacteria) for efficient and faster removal of heavy metals.

Oves et al. (2017) isolated bacterial strains from chickpea rhizosphere and molecularly characterized as *Ensifer adhaerens* (OS3). This strain was studied for its heavy metal tolerance, bioremediation, and phosphate solubilizing ability.

The strain was resistant to a wide variety of metals such as Cd (250 μg mL^{-1}), Cr (500 μg mL^{-1}), Zn (800 μg mL^{-1}), Cu (800 μg mL^{-1}), and Ni (1000 μg mL^{-1}). At 34 °C and pH 7.0, OS3 strain accumulated 95% of Ni and 74% of Pb and reduced 200 μg mL^{-1} of chromium in 96 h. Apart from the heavy metal bioremediating ability, the strain was also known to solubilize inorganic phosphate (303 μg mL^{-1}) and produce indole acetic acid (IAA), siderophores, hydrogen cyanide (HCN), and ammonia. All these properties of OS3 made this organism a choice for bioremediation and for plant growth promotion.

In symbiotic association, algae produce oxygen during its photosynthetic cycle and oxygen acts as a key electron acceptor in metal degradation for the heterotrophic bacteria. On the other hand, bacteria produce carbon dioxide from its metabolic activity and secretes many growth factors required for photoautotrophic growth of algae (Luo et al. 2014). Self-oxygenated algae and bacteria consortia system is an ideal self-sustaining system. It reduces the cost of technology as conventional methods need continuous oxygen supply (Subashchandrabose et al. 2011).

In bacteria-algae consortia, synergistic effect and different metabolic activities play a crucial role in reducing the pollution; sometimes it would not be possible with a single strain (Zeraatkar et al. 2016). The bacteria and algal strains in consortia are diverse in metabolic activities and have wide tolerance to fluctuating environmental conditions and pathogen invasion (Subashchandrabose et al. 2011). The algae provide O_2-stable habitats to bacteria and enhance the bioavailability of pollutants for better bacterial degradation (Gutierrez et al. 2012), thereby enhancing the rate of removal than single species (Luo et al. 2014). A continuous flow-through column containing bacteria and algal co-culture had removed Cu and Cd, up to 80% and 100%, respectively (Subashchandrabose et al. 2011).

The photo-rotating biological contractors holding biofilm with immobilized algae (*Ulothrix* sp.) and bacterium removed 20–50% of a large variety of metals in 10-day incubation period and the removal rate was in the following order: Cu > Ni > Mn > Zn > Sb Se > Co > AI (Orandi et al. 2012). The various types of bacteria and algae used for bioremediation of heavy metals are shown in Table 4.

6.5.1 Future Prospects

The challenge in outdoor field application of consortia (bacteria and algae) is real-time monitoring of healthy mixed populations and maintaining optimum ratio of bacteria and algae. This would require various strategies and different types of bioreactor design and operation. Currently the knowledge on algae-bacteria interactions is very limited; it is necessary to identify and study efficient bacteria to co-culture with algae to develop a robust consortia system to mitigate the different heavy metal problems in the environment at large scale.

Table 4 List of bacteria and algal consortia used for different heavy metal removal

Microalgae	Bacterium	Metal removal efficiency	References
Spirulina platensis	Sulfate-reducing bacteria	Cu 79.2% (500 mg L^{-1}) Zn 88% (500 mg L^{-1}) Iron 100% (500 mg L^{-1})	Rose et al. (1998)
Chlorella sp. *Scenedesmus obliquus*	*Rhodococcus* sp. *Kibdelosporangium aridum*	Cu 62% (0.04 mg L^{-1}) Ni 62% (0.21 mg L^{-1}) Zn 90% (0.1 mg L^{-1})	Safonova et al. (2004)
Chlorella sorokiniana (Biomass)	*Ralstonia basilensis (biomass)*	Cu 57.5% (20 mg L^{-1}), pH 5.0	Munoz et al. (2006)
Spirogyra hyaline		Cd, Hg, Pb, As, and Co	Kumar and Cini (2012)
	Alcaligenes sp. *Pseudomonas* sp. *Moraxella* sp.	Cd	Springael et al. (1993)

6.5.2 Conclusion

Heavy metal pollution is a global concern. The conventional treatment methods are costly and are a source of secondary pollution. Bioremediation is an eco-friendly approach that uses heavy metal-resistant microorganisms to mitigate environmental contamination. Since industrial effluents contain many heavy metals and other pollutants, the bioremediation of these wastes could be difficult to treat by a single microbe. In nature the algae and bacteria coexist along with their natural ability to remove heavy metals. The bioremediation process involving consortia of bacteria and algae could be an efficient system in reducing the pollution. Symbiotic associations of algae and bacteria would provide robustness against environmental fluctuations, stability to the associate member, and mutual metabolite sharing. The synergy between bacteria and algae in consortia approach would help in faster removal of heavy metals by the multiple mechanisms than single organisms.

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Recent Advances in Aluminum Phytotoxicity

Filiz Vardar

Contents

1 Introduction

Aluminum (in American English, aluminium in British English) is the third most abundant element following oxygen and silicon, while its oxide is the fourth among the most common compounds in the earth's crust. Aluminum (Al) is also the most abundant metal on the planet. Al is dense in the outer 16 km of earth's cortex constituting about 8.1% by mass. Naturally, Al never occurs in the metallic form because of its chemical activity; it is found in chemical compounds with other elements like bauxite. To remove Al from natural ores, it must first be reduced. Al is considered as an active metal reacting with concentrated acids and alkalis (Sade et al. 2016; Li et al. 2016).

The trivalent Al has three oxidation states. The most common oxidation state of Al is +3 and it reacts rapidly with the oxygen in the moist air to form aluminum oxide (Al_2O_3-alumina). Al_2O_3 is the refractory oxide of Al existing in bauxite. Occasionally, the oxidation state of +2 and +1 exists as aluminum monoxide (AlO) and aluminum hydride (AlH_3), respectively. The Al^{3+} ion can be stabilized by hydration, and the octahedral ion $[Al(H_2O)_6]^{3+}$ occurs both in aqueous solution and in several salts (Roesky and Kumar 2005; Li et al. 2016).

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It has been known that Al is also the most widely used metal in the industrial world after iron (Table 1). The large-scale (28%) use of Al is in the transportation industry. Packaging follows it by 23%. Because Al can be melted and reused, or recycled, it is ideal for foil, beer and soft drink cans, paint tubes, and containers for home products such as aerosol sprays. 14% of Al goes into building and construction such as windows and door frames, screens, roofing, and siding, as well as the construction of mobile homes and structural parts. The remaining 35% of Al is used in electrical wires and appliances due to being an excellent conductor, automobile engines, heating and cooling systems, bridges, vacuum cleaners, kitchen utensils, garden furniture, heavy machinery, and specialized chemical equipment (http://www.chemistryexplained.com/elements/A-C/Aluminum.html).

The widespread presence in earth crust and prevalent use of bioavailable Al may have immense and far-reaching implications for the health of humans and animals.

Table 1 Aluminum complexes used in industries (http://www.chemistryexplained.com/elements/A-C/Aluminum.html)

Al complex	Chemical formula	Area of usage
Aluminum ammonium sulfate	$Al(NH_4)(SO_4)_2$	• Mordant • Water purification and sewage treatment • Paper production • Food additive • Leather tanning
Aluminum borate	$Al_2O_3B_2O_3$	• Production of glass and ceramics
Aluminum borohydride	$Al(BH_4)_3$	• Additive in jet fuels
Aluminum chloride	$AlCl_3$	• Paint manufacture • Antiperspirant • Petroleum refining • Production of synthetic rubber
Aluminum fluorosilicate	$Al_2(SiF_6)_3$	• Production of synthetic gemstones, glass, and ceramics
Aluminum hydroxide	$Al(OH)_3$	• Antacid • Mordant • Water purification • Manufacture of glass and ceramics • Waterproofing of fabrics
Aluminum phosphate	$AlPO_4$	• Manufacture of glass, ceramics, pulp and paper products • Cosmetics • Paints and varnishes • In making dental cement
Aluminum sulfate, or alum	$Al_2(SO_4)_3$	• Manufacture of paper • Mordant • Fire extinguisher system • Water purification and sewage treatment • Food additive • Fireproofing and fire retardant • Leather tanning

In fact, much evidence shows that Al seems to be toxic to all forms of life on earth, and where it also appears in terrestrial biochemistry, it is invariably deleterious (Exley 2009; Shaw and Tomljenovic 2013).

2 Aluminum Toxicity in Plants

Considering the plants, Al is one of the abiotic stress factors. It is definite that anthropogenically released and/or naturally existing Al can solubilize and be absorbed by plants at low pH (acid) soils constituting one of the major plant growth-limiting factors. It has been known that potential farmable lands (approx. 67%) have acid soil worldwide (Abate et al. 2013; Ma et al. 2014). Al exists as a nontoxic complex in neutral or weakly acidic soils; however, when the complex Al is solubilized it turns to phytotoxic forms in acid soils. The most phytotoxic and dominant form is $Al(H_2O)_6^{3+}$ and dissolves to Al^{3+} which can be absorbed by plant roots (Matsumoto 2000; Vardar and Ünal 2007). It has been stated that solubilized Al presents in the range of 10–100 μM in acid soils affecting adversely the plant growth and development within a few minutes (Ciamporová 2002; Vitorello et al. 2005; Abate et al. 2013). Absorbed Al interacts with apoplasmic (cell wall), plasma membrane, and symplasmic (cytosol) targets. Al ions are penetrated from roots primarily and only a small proportion may be taken up through leaves (Kochian et al. 2005; Singh et al. 2017).

At the whole plant level, toxic Al affects adversely their anatomical and physiological structure such as chlorosis, reduction in leaf number, reduced photosynthesis, necrosis, and retardation of root growth. It has been widely known that roots are the first target of Al toxicity which have direct contact with rhizosphere. Al-induced root growth inhibition restrains the uptake of soil water and essential minerals leading to reduction in crop quality (Delhaize et al. 2004; Singh et al. 2017; Vardar et al. 2018). Root apex is the foremost region with regard to Al toxicity. As a first target, root apex plays a critical role in Al penetration and accumulation. This region absorbs more Al than the upper parts of root resulting in morphological alterations of root apices such as swelling, cracking, and appearing to be stubby and stiff (Matsumoto 2000; Vardar et al. 2006). It has also been visible that branching and root hair occurrence reduce significantly (Ciamporová 2002; Vardar et al. 2011). Several researches revealed that Al has detrimental effects reacting with different subcellular regions performed in different species and also varieties.

2.1 Cell Wall

Considering the cell structure, cell wall is the primary target of Al ions. It has been identified that Al binds and accumulates in the apoplasm in the range of 30–90% in root cortex cells (Rengel and Reid 1997; Vardar et al. 2011). Pectin matrix which has negatively charged carboxylic groups is the first Al-binding site in the cell wall (Chang et al. 1999; Singh et al. 2017). It has also been known that Al reacts with apoplasmic face of plasma membrane. After Al-cell wall interaction, Al translocates to plasma membrane and symplasm (Schmohl and Horst 2000). Researchers suggested that Al accumulation degree is in direct correlation with pectin content and dissociation of carboxylic and hydroxylic groups of the pectin (Godbold and Jentschke 1998; Ahn and Matsumoto 2006). Strong binding and accumulation of Al alter the structural and mechanical properties of cell wall causing reduction in mechanical extensibility causing cracked and unoriented root growth (Kochian et al. 2005).

Al accumulation in cell wall discomposes the stability of other cations such as Ca^{2+} which is responsible for the strength of cell wall. This disturbance causes callose (β-1,3-glucan) synthesis and accumulation between cell wall and plasma membrane being one of the significant markers of Al toxicity (Tabuchi and Matsumoto 2001; Vardar et al. 2011; Ünal et al. 2013). Although callose may collaborate root to cope with Al toxicity by blocking the plasmodesmata, it also blocks the movement of water and minerals causing reduction of nutrient uptake (Singh et al. 2017; Vardar et al. 2018).

Eventually, Al reaction in cell walls causes reduced extensibility, disrupted growth orientation, callose formation, and accordingly restriction of water and mineral nutrient uptake across the plasma membrane (Kochian et al. 2005).

2.2 *Plasma Membrane*

Plasma membrane is the external barrier of the cell and it regulates the ion traffic. Negatively charged membrane displays strong interaction with $A1^{3+}$ (Kinraide et al. 1998). Plasma membrane-Al reaction alters the structure and function of membrane causing disruption in the cellular homeostasis (Kochian et al. 2005). It has been revealed that $A1^{3+}$ may interact with both phospholipids and proteins leading to lipid peroxidation in plasma membrane. Researchers revealed that the severity of Al toxicity causes to break the plasma membrane integrity (Vitorello et al. 2005; Panda et al. 2009; Singh et al. 2017). Lipid peroxidation also causes highly toxic free radical generation and accumulation (Panda et al. 2009).

Al has greater affinity than other cations such as Ca^{2+} and Mg^{2+} during competing for the choline head of phosphatidylcholine. This situation culminates in Al-induced positively charged bridges between head groups of the phospholipid layer and displacement of other cations (Bhalerao and Prabhu 2013; Singh et al. 2017). The positively charged layer restricts cation motion, but increases anion movement altering membrane electrochemical potential (Nichol et al. 1993). As we have stated above Al-induced cation alteration, principally Ca^{2+} displacement, also triggers callose synthesis (Gupta et al. 2013). Callose also inhibits intercellular transport through plasmodesmatal plugs (Sivaguru et al. 2000). Alterations in cation uptake of essential ions such as Ca^{2+}, K^+, Mg^{2+}, and NH_4^+ also cause nutrient imbalances (Pineros and Kochian 2001; Singh et al. 2017).

2.3 Cell Signaling and Cytoskeleton

Several researchers stated that Al stress affected signal transduction pathway adversely mediated by secondary messengers due to imbalance of Ca^{2+} and pH homeostasis (Jones and Kochian 1997; Ma et al. 2002; Singh et al. 2017). In plasma membrane Al prefers to react with specific lipids which are important signaling molecules such as G proteins (guanine nucleotide-binding proteins) and a phosphatidylinositol-4,5-diphosphate (PIP2)-specific phospholipase C commonly (He et al. 2015). Besides, Al stress decreases inositol-1,4,5-triphosphate (IP3) amount in the plasma membrane (Rengel and Elliott 1992). After Al reaction, signaling pathways are interrupted in the cell.

Cell cytoskeleton including microtubules, microfilaments, and intermediate filaments is also one of the potential targets of Al ions. Al causes disruption in cytoskeletal dynamics which has a critical importance during cell-wall biosynthesis, cell growth, and cell division. It has been revealed that Al-induced disruption of microtubule and actin filament results in lateral cell swelling (Frantzios et al. 2001; Sivaguru et al. 2003). It has been suggested that Al disruption in cytoskeleton occurs either through direct interaction with cytoskeletal elements or through alteration in signaling pathway (Sivaguru et al. 1999). Protein phosphorylation-dephosphorylation and mitogen-activated protein kinase (MAPK) cascade which take charge during signal transduction are also reorganized by Al ions (Matsumoto 2000; Osawa and Matsumoto 2001; Singh et al. 2017). This interaction impairs the signal transduction pathway causing chaos in the cell.

2.4 Genotoxicity

Several researches reveal that Al has genotoxic impact and long-term Al exposure causes adverse effects on DNA composition and replication due to more rigid double-helix and chromatin structure (Vitorello et al. 2005; Panda et al. 2009; Gupta et al. 2013). It has been observed that Al ions decrease cell viability and mitotic index and increases chromosomal aberrations which are associated with Al-induced disturbance in tubulin polymerization-depolymerization. Tubulin disturbance limits the movement of chromosome on mitotic spindle causing chromosome laggards, bridges, micronuclei, and c-mitosis under Al stress (Frantzios et al. 2000; Vardar et al. 2011). It can also be considered that Al exposure may decrease the frequency of S-phase cells inducing delay in M phase (mitotic division) (Jaskowiak et al. 2018). Grabski and Schindler (1995) showed that Al has greater affinity to nucleoside triphosphates much more than Mg^{2+}. Hence, Al prefers to interact with DNA than histone proteins at first. Besides, several researches revealed that Al exposure may cause double-strand DNA breaks even at 15 min (Vardar et al. 2015, 2016). Recent studies also revealed that Al ions cause DNA methylation and polymorphism of LTR retrotransposons (Guo et al. 2018; Taspinar et al. 2018).

2.5 *Oxidative Stress and Programmed Cell Death*

Al toxicity stimulates generation of reactive oxygen species (ROS) leading to oxidative stress in plants. Lower concentrations of ROS have a role as signaling molecules; however, higher concentrations regress the balance between antioxidant machinery and ROS detoxification. Overproduction of ROS ($^{\cdot}O_2^-$, $^{\cdot}OH$, HO^-, H_2O_2) is generated in mitochondria, chloroplast, and peroxisomes causing imbalance of antioxidant enzyme, lipid peroxidation, protein denaturation, carbohydrate oxidation, pigment breakdown, and DNA damage (Sharma and Dubey 2007; Gupta et al. 2013; Vardar et al. 2018).

Phytotoxic levels of ROS also trigger programmed cell death (PCD) in plants. It has been suggested that ROS weakens the binding strength of cytochrome c (cyt c) through oxidation of cardiolipins in the inner mitochondrial membrane and reduces mitochondrial membrane potential ($\Delta\Psi m$) inducing cytochrome c release to the cytoplasm (Williams et al. 2014). Besides cytochrome c release amplifies more ROS generation and triggers vacuolar processing enzyme (VPE) activity. Although there are some studies concerning Al toxicity and PCD (Table 2), more detailed studies are needed to clarify the Al-induced PCD mechanism.

3 Al Tolerance Mechanisms

Al has the ability to make stable complexes with oxygen donor ligands; thus Al chelating with root exudates plays a critical role in the prevention of phytotoxic Al uptake by roots (Barceló and Poschenrieder 2002). It has been evidenced that Al chelating mechanism is performed by mucilage formation, organic anion efflux, phosphate secretion, and secondary metabolite production from tolerant root apices (Miyasaka and Hawes 2001; Ma et al. 2001; Ofei-Manu et al. 2001; Vardar and Ünal 2007; Singh et al. 2017). Whereas tolerant plants may use different types of Al exclusion strategies, organic anion efflux plays a central role in the exclusion of Al. Several genetic and molecular approaches concerning organic acid release were reported in different plant species (Ma et al. 2001). Al chelation by organic acids decreases or prevents its uptake through apoplasm and symplasm. Type of organic acids secreted by roots varies depending on Al-tolerant plant species. It has been reported that malate, citrate, and oxalate are the most commonly encountered organic secretions (Magalhães et al. 2007; Ryan et al. 2009). Researches revealed that organic acid exudation is activated by Al exposure rapidly suggesting a transporter located in the plasma membrane of tolerant roots (Kochian et al. 2005).

Whereas organic acid exclusion from roots and Al chelation in the rhizosphere appear to be the most common, several species tolerate Al toxicity by internal or symplastic detoxification after Al uptake into the root or shoot cells. This situation was first attained in Al-accumulating plant root, shoot, and leaf such as tea (*Camelia sinensis*), buckwheat (*Fagopyrum esculentum*), and Hydrangea (*Hydrangea macrophylla*).

Table 2 Recent studies concerning Al-induced PCD

Plant material	Al concentration	PCD signs	References
Hordeum vulgare	0.1–50 mM	DNA fragmentation (0.1–1 mM) Necrotic DNA smear (10–50 mM)	Pan et al. (2001)
Allium cepa	1–200 µM	Breaks in DNA	Achary et al. (2008)
A. cepa	200–800 µM	DNA damage	Achary and Panda (2009)
Arabidopsis thaliana	0.5 mM	Caspase-3-like activity Loss of MTP Swelling of mitochondria ROS generation	Li and Xing (2011)
H. vulgare	2.5, 5, 10 mM	DNA damage	Achary et al. (2012)
Nicotiana tabacum	0–150 µM	VPE activity Alterations in vacuole	Kariya et al. (2013)
Arachis hypogaea	100 µM	ROS burst Upregulation of *Rboh* and *COX* expression MPTP opening Decreased ΔΨm Cyt c release Caspase-3-like protease activity DNA fragmentation	Huang et al. (2014)
A. hypogaea	20, 100, 400 µM	ROS production MDA increase Reduction of mitochondrial Ca concentration Opening of MPTP Collapse of ΔΨm Cyt c release	Zhan et al. (2014)
H. vulgare *Secale cereale* *Triticosecale wittmack* *Avena sativa*	100 µM	Caspase-3, -8, and -9-like activities	Aytürk and Vardar (2015)
Triticum aestivum *S. cereale* *T. wittmack*	100 µM	DNA damage	Vardar et al. (2015)
H. vulgare *S. cereale* *T. wittmack* *A. sativa*	100 µM	DNA fragmentation	Vardar et al. (2016)
A. hypogaea	100 µM $AlCl_3$	Caspase-1, -2, -3, -4, -5, -6, -8, and -9 activities	Yao et al. (2016)
Nicotiana tabacum	50 µM	Increase in gene expression of VPE1a and VPE1b	Kariya et al. (2018)
H. vulgare	5–60 µM	DNA fragmentation	Jaskowiak et al. (2018)

MTP mitochondrial transmembrane potential, *MPTP* mitochondrial permeability transition pore

Internal detoxification consists of Al chelation with organic ligands in cytosol and their transfer to the vacuole for deposition (Kochian et al. 2004; Delhaize et al. 2012). Although most of the plants prefer only organic acid exudation or internal detoxification, some species such as *Pinus taeda* make use of both of the mechanisms to protect itself from Al toxicity (Nguyen et al. 2003; Nowak and Friend 2005).

4 Aluminum Tolerance Genes in Plants

Many plant species vary considerably in their ability to tolerate the toxic Al concentrations via efflux of organic anions such as malate, citrate, and oxalate from roots. Al tolerance has a strong correlation with genotype-dependent efflux capacity of organic anion and exclusion of Al once it enters cytosol (Kochian et al. 2004; Hiradate et al. 2007; Delhaize et al. 2012). Sasaki et al. (2004) isolated a gene controlling the Al-dependent efflux of malate from *Triticum aestivum* (wheat) named *TaALMT1* (*Triticum aestivum* aluminum-activated malate transporter 1). *TaALMT1* (formerly named *ALMT1*) encodes a hydrophobic protein (anion channel) localizing in the plasma membrane of root cells (Yamaguchi et al. 2005; Ligaba et al. 2006). ALMT protein family has 5–7 membrane-spanning regions in the N-terminal half of the protein and a long C-terminal tail (Delhaize et al. 2004, 2012). Researchers revealed that *TaALMT1* expression in Al-tolerant genotypes of wheat is 5- to 10-fold higher than in Al-sensitive genotypes (Sasaki et al. 2004; Raman et al. 2005). Subsequent analyses revealed that specific variations in diverse bread wheat genotypes could be classified into seven patterns, type I to type VII (Sasaki et al. 2006; Garcia-Oliveira et al. 2014). After the discovery of *ALMT1* in wheat, *Arabidopsis ALMT1* members were identified as *AtALMT1*, and similarly their homologs characterized in rape (*BnALMT1* and *BnALMT2*), soybean (*GmALMT1*), and rye (*ScALMT1*). All of them share similar functional characteristics that induce malate exudation in Al tolerance (Hoekenga et al. 2006; Ligaba et al. 2006).

Further studies revealed that another gene responsible for citrate exudation in response to Al toxicity exists in barley (*HvAACT1-Hordeum vulgare* aluminum-activated citrate transporter 1) which belongs to *MATE* (multidrug and toxic compound extrusion) gene family (Furukawa et al. 2007). Besides, *SbMATE* gene was also identified in *Sorghum bicolor* responsible for citrate transporter in response to Al toxicity (Magalhães et al. 2007).

It has been known that tolerant genotypes within species have significantly much more organic acid expression than sensitive genotypes. The extra expression is due to a series of *cis* mutations in the promoter of *TaALMT1* in wheat (Sasaki et al. 2006; Ryan et al. 2010). Raman et al. (2008) revealed that the promoter region is more polymorphic than coding region in *TaALMT1* and several alleles have accurate tandem repeats (Ryan et al. 2010). Besides, several examples indicated that transposable elements are able to alter the level and localization of gene expression during enhancing Al tolerance (Morgante et al. 2007; Delhaize et al. 2012).

In wheat the major Al tolerance locus was identified on chromosome 4DL (Luo and Dvořák 1996; Raman et al. 2008) and subsequently on chromosome 4BL responsible for phenotypic variation in citrate efflux (Ryan et al. 2009) suggesting that citrate is the secondary organic acid after malate in Al tolerance. Following molecular studies in different cultivars of wheat revealed that multiple genetic loci on the chromosome arms of 2DL, 3DL, 4BL, 4DL, 5AS, 6AL, 7AS, and 7D are very critical in Al tolerance mechanism (Aniol and Gustafson 1984; Aniol 1990; Papernik et al. 2001). However, it is still not clear that whether all of these loci are included in Al tolerance. Recently Al tolerance-related loci have been identified on different chromosomes in different plant species (Ryan et al. 2009; Boff et al. 2019).

Early studies suggested that Al resistance in wheat is driven by a single major genetic locus with different alleles inducing different degrees of Al tolerance (Campbell and Lafever 1981). Monogenic inheritance with multiple alleles was also identified in barley, maize, sorghum, pea, chickpea, and oat (Singh and Choudhary 2010; Singh and Raje 2011; Castilhos et al. 2011; Delhaize et al. 2012). However, subsequent microarray studies revealed the complexity of the genetic control of Al tolerance. Besides, most of the identified genes probably express response to Al stress rather than Al tolerance (Goodwin and Sutter 2009; Delhaize et al. 2012). According to the several researches in wheat root tips different genes expressed high amounts correlating with Al tolerance such as *ALMT1*, ent-kaurenoic, β-glucosidase, lectin, histidine kinase, pyruvate dehydrogenase, alternative oxidase, galactonolactone oxidase, and phosphoenolpyruvate carboxylate. These results suggest that Al tolerance can be co-regulated by multiple genes with diverse functions in plants in addition to *ALMT1* (Guo et al. 2007; Houde and Oury 2008).

In conclusion, Al toxicity is a widespread problem in industrial regions and acidic soils limiting crop productivity in the world. It has been known that the severity of Al toxicity is due to plant genotype, cell/tissue type, types of chelators, concentrations of other cations, and pH (Kinraide and Parker 1987). Since Al toxicity and tolerance mechanism and also Al-detoxifying mechanisms need to be clarified with more detailed studies, in this chapter, we reviewed recent information concerning physiological and molecular effects of Al toxicity and Al tolerance mechanism. The intensive researches on gene-based mechanisms of Al toxicity and tolerance may help to develop Al-tolerant varieties or transgenic to enhance the crop quality under Al toxicity.

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Role of Biochar in Heavy Metal Toxicity in Plants

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Contents

1 Introduction

Heavy metals (HMs) are considered extremely dangerous environmental pollutants which are highly toxic and carcinogenic and have the potential for bioaccumulation, even at very low concentrations. They have a potential to cause serious threats to both human and environmental health (Zou et al. 2016). Recently, various kinds of conventional technologies are used for remediation of HM phytotoxicity in plants, which help in maintaining public safety as well as ecological sustainability. These conventional technologies consist of chemical precipitation (Peligro et al. 2016), membrane removal (Fu and Wang 2011), ion exchange (Huang et al. 2016), chelation/complexation (Wang et al. 2012), flotation (Kaya 2016), and phytoremediation

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(Fu and Wang 2011; Ma et al. 2016). However, these methods are too expensive and produce harmful secondary wastes such as persistent organic pollutants and volatile organic compounds, and therefore these techniques cause huge environmental risks (Vuković et al. 2010). There is therefore crucial demand for cost-effective as well as environmentally friendly methods for effective removal of HMs. Adsorbent materials are commonly used for HM remediation. Among these adsorbent materials, biochar (BC) is gaining attention currently (Li et al. 2010). It is the carbon-rich product obtained mainly from the wood, manure, or leaves and is heated in a closed container with little or no available air. In technical terms, biochar is considered as a product of thermal decomposition of organic material under limited supply of oxygen (O_2), and at relatively low temperatures (>700 °C). BC can be made from a vast variety of waste biomass feedstock residues (straw, husk, sawdust, sludge, distillery grains, kitchen waste, tea residue, etc.), increasing its potential production sources, resulting in reduced production costs and energy requirements (Zheng et al. 2010). This chapter deals with the heavy metal toxicity protection using biochar.

2 Heavy Metal Phytotoxicity

Plants have to combat various adverse environmental conditions in their lifetime and presence of excessive heavy metals in the soils and irrigation waters is one of the major causes of toxicity in plants. Heavy metal pollution holds a high significance because of environmental, nutritional, ecological, and evolutionary reasons (Kalaivanan and Ganeshamurthy 2016). Metal ions have been shown to be harmful to plants at even trace levels if they are nonessential, while essential metals become toxic at elevated concentrations. Usually, these are involved in producing reactive oxygen species (ROS) by undergoing either Haber-Weiss or Fenton reactions. The end products of these reactions are hydroxyl radicals that are major cause of oxidative damage in cells (Halliwell and Gutteridge 1990; Kalaivanan and Ganeshamurthy 2016). These radicals, along with other ROS, have damaging effects on vital biomolecules. Damage to DNA is one of the severe effects of ROS that have implication on the growth and development of the organism. It occurs by either breakage of double bonds, removal of nitrogen bases, or breakage of sugar phosphate backbone (Yadav and Sharma 2016). Lipids are major constituents of biomembranes and hence are essential part of cell and cell organelles. Polyunsaturated fatty acids present in the membranes are essential for maintaining their integrity but at the same time these easily undergo peroxidation when attacked by ROS that leads to the formation of aldehydes (Møller et al. 2007; Gill and Tuteja 2010). This changes the composition and properties of membranes and makes the membranes leaky due to alterations in the channels, receptors, membrane proteins, etc. (Halliwell 2006; Yadav and Sharma 2016). Proteins, when coming in contact with ROS, are susceptible to both reversible and irreversible changes leading to major alterations in metabolic activities (Choudhury et al. 2017). ROS affect proteins both in pre-translational and posttranslational manner. Sulfonylation is one of the posttranslational modifications in which oxidation of sulfhydryl groups takes place. It leads to conformational changes in the protein structure or activities of enzymes due to the formation of disulfide bonds in cysteine residues or by oxidation of methionine leading to the formation of Met-sulfoxide (Choudhury et al. 2017). Proteins also undergo another type of oxidation called carbonylation in which arginine, histidine, lysine, proline, and threonine undergo oxidation leading to an irreversible change (Shacter 2000). Mitochondrial proteins are found to be highly susceptible to carbonylation and such proteins lead to cellular dysfunction and finally proteasomal complexes degrade these damaged proteins (Yadav and Sharma 2016).

The general effect of heavy metals is first measured in the form of plant growth inhibition. Root system of the plants is the first organ which comes in contact with heavy metals and hence shows initial symptoms of toxicity (Appenroth 2010). These symptoms include reduced length of primary root system, reduction in secondary growth and root hair surface, and increased die back (Lux et al. 2010; Chen and Yang 2012; Sharma 2012; Anjum et al. 2013; Feleafel and Mirdad 2013). Heavy metal stress also results in altered water absorption by root system that reduces its water content (Rucińska-Sobkowiak 2016). Salts of heavy metals have the ability to cause changes in osmotic potential of soils that can restrict water absorption. However, before reaching that threshold limit, the metal ions show toxic symptoms on roots. Hence, any changes in water relations are an indirect effect of metal ions on the root morphology and anatomy (Rucińska-Sobkowiak 2016).

Photosynthetic mechanism is negatively affected by stress caused by heavy metals. These may affect the process indirectly by causing stomatal closure and leading to reduced photosynthetic and transpiration rates (Aggarwal et al. 2011). The direct effects of heavy metal stress include their binding to the active sites of several biomolecules. The structure of thylakoid membranes is disturbed by heavy metals which causes alteration in light reactions (Aggarwal et al. 2011). It is, however, usually correlated to excessive lipid peroxidation and protein carbonylation that disturbs the membrane structure (Aggarwal et al. 2011). Biosynthesis of essential photosynthetic pigment chlorophyll is also known to be disrupted by heavy metals. Two key enzymes of the pathway, d-aminolevulinic acid (ALA)-dehydratase) and protochlorophyllide reductase, are highly sensitive to heavy metal toxicity (Vangronsveld and Clijsters 1994). Also, the electron transport chain in photosynthetic pathway and the photosystems are affected negatively by heavy metal stress. Most of the metals are potential inhibitors of photosystem II due to which light reactions get disrupted and ultimately it affects the entire pathway (Prasad and Strzalka 1999; Aggarwal et al. 2011).

3 Factors Involved in Metal Toxicity Mitigation

The plants, in response to stress induced by heavy metals, defend themselves to survive against the toxicity. Defense mechanisms become activated which help in either avoiding or tolerating the ill effects of heavy metals. The first line of defense against heavy metals is formed by physical barriers such as increase in the thickness of cuticle and cell walls, presence of trichomes, and enhanced mycorrhizal association (Emamverdian et al. 2015). If heavy metal ions surpass these barriers and gain entry in cells, the cellular defense of the plants becomes active. Several defense-related biomolecules are synthesized which help in plant protection. Phytochelatins are specialized molecules that have the ability to detoxify toxic metal ions due to their affinity to bind them. These thiol-rich compounds having peptides of low molecular weights are of widespread occurrence from fungi to plants (Vatamaniuk et al. 2001; Bundy et al. 2013; Emamverdian et al. 2015). They are produced in cytosol and once they bind to metal ions, they are transported to vacuole (Manara 2012; Song et al. 2014). Metallothioneins are another group of proteinaceous compounds which are cysteine rich and have low molecular weights (Emamverdian et al. 2015). As compared to phytochelatins, metallothioneins have affinity of more number of metal ions and hence these are highly effective (Yang and Chu 2011). Their significance in plant defense also becomes imperative due to their roles in scavenging ROS, plasma membrane repair and maintenance of cellular redox, and repair of DNA (Wong et al. 2004; Mishra and Dubey 2006; Macovei et al. 2010; Grennan 2011).

Proline is an important biomolecule which is not only a proteinogenic amino acid but also acts as a compatible solute. It is also an important component of cell wall, helps in scavenging free radicals, and aids in stabilizing several macromolecules (Seregin et al. 2003; Pavlíková et al. 2007; Zarei et al. 2012). Plants, when facing abiotic stress, usually show an enhanced content of proline. Such a response enhances the adaptation of plants and their recovery from stresses (Fidalgo et al. 2013). As the chief function of proline is osmoregulation, it was proposed by Clemens (2006) that heavy metal stress does not directly induce its accumulation but it is an indirect effect of disorder in water balance that occurs due to such stress. Proline is involved in detoxification of hydroxyl radicals, and singlet oxygen species (Tripathi and Gaur 2004). It is also involved in the protection of antioxidative enzymes and functions in enhancing their activities (Mourato et al. 2012). It also regulates cellular pH and redox levels, protects chlorophyll and proteins, and chelates heavy metals (Mishra and Dubey 2006; Rastgoo et al. 2011). Antioxidative defense system of plants, that comprises enzymes and antioxidants, is mainly involved in overcoming excessive ROS production due to heavy metal stress. Several enzymes such as superoxide dismutase, peroxidases, catalase, ascorbate peroxidase, and glutathione-S-transferase in coherence with antioxidants such as glutathione and ascorbic acid convert superoxide radicals and hydrogen peroxide to water and oxygen (Singh et al. 2015). Both types of antioxidants are able to detoxify diverse types of ROS and protect the cells from oxidative stress.

Apart from preexisting defense mechanisms in the plant system, several workers have used various other strategies to enhance the tolerance of plants against heavy metal stress. Exogenous usage of phytohormones such as brassinosteroids, salicylic acid, and jasmonic acid has been reported to boost the defense system of plants and combat the stress. Development of transgenic plants with increased tolerance levels has also come into practice but such strategies are only utilized where the plants have to be used for phytoremediation purposes (Koźmińska et al. 2018). Several soil reclamation methods have also been used to reduce the uptake of heavy metals. Among such strategies, usage of **biochar** has also become popular in recent times. It is produced from carbonization of organic materials such as residues of wood, manure, paper sludge, and some industrial wastes (Lucchini et al. 2014). Its large surface area provides it a property to adsorb heavy metals as well as organic and inorganic pollutants (Zhang et al. 2013). It has also been reported to enhance the retention of plant nutrients in soil and decrease leaching losses (Al-Wabel et al. 2014). The metal/metalloid contaminants in the soil, in their cationic or anionic form, interact with the charges on the biochar surface and ultimately bind to them (Sun et al. 2018). This leads to immobilization of metal/metalloid ions, thereby lowering the solubility of these ions (Sun et al. 2018). The pH of the soils is also one of the major factors that control the solubility of metal ions and biochar has the ability to alter the pH and this changes the solubility of metal/metalloid ions (Sun et al. 2018).

4 History of Biochar

There is a long and complex history of biochar, which is rooted in indigenous soil practices in the Amazon known as Terra Preta de Índio (also known as Amazonia Dark Earths) (Mann 2002; Marris 2006; Johannes Lehmann and Stephen Joseph 2017). This *terra preta* soil is highly fertile and is dark in color. This soil is considered as the supportive of agricultural needs of the Amazonian people. Analyses of this soil revealed the high concentrations of organic matter, like animal and plant remains (bones, fish, and manures) and charcoal. It has also been found that the productivity of *terra preta* is due to its high nutrient retention power and also neutral pH in areas where soils are found to be acidic (Lehmann and Joseph 2007). Moreover, it was also reported that terra preta exists in the inhabited areas only, suggesting its anthropogenic origin.

Many theories were proposed in order to explain the techniques involved in the formation of this highly fertile soil. A front runner among these theories is the suggestion that ancient techniques of slash and char are responsible for the dark soil. Similar to slash-and-burn techniques, slash-and-char involves clearing vegetation within a small plot and igniting it, but only allowing the refuse to smolder (rather than burn). Combined with other biomass and buried under a layer of dirt, the smoldering char eventually forms *terra preta* (Ring 2007; Talberg 2009).

It was thought and hoped that when the processes involved in the formation of terra preta are understood, this knowledge can be utilized to recreate terra preta, improve the fertility of soil, increase carbon sequestration, and decrease carbon emissions (Teixeira et al. 2009). By gathering all these information, attempts were made to recreate terra preta; these attempts first led to the Terra Preta Nova Project but later inspired technological developments that gave rise to the concept of biochar (Maia et al. 2011).

Currently, biochar is considered as a new technology that is believed to be able to deliver multiple benefits, including enhanced soil fertility, energy production, and carbon sequestration through biofuels, and waste management (Bezerra et al. 2016).

5 Physicochemical Properties of Biochar

5.1 Physical Properties of Biochar

Surface properties, including surface area, charge density, pore structure, and distribution, are important characteristics as they influence the essential functions for retention capacity of water, nutrients, and microbial activity. The physical features of biochars are important in soil processes. Lehmann and Joseph (2009) showed that operating parameters, including processing heating rate, highest treatment temperature (HTT), pressure, reaction residence time, reaction vessel (orientation, dimensions, stirring regime, catalysts, etc.), pretreatment (drying, comminution, chemical activation, etc.), and flow rate of ancillary inputs, all influence the resultant physical properties of biochar made with any biomass feedstock. Various physical properties are discussed below:

5.1.1 Density and Porosity

Two types of density of biochars can be studied: the solid density and the bulk or apparent density. Solid density is the density on a molecular level, related to the degree of packing of the C structure. Bulk density is that of the material consisting of multiple particles and includes the macroporosity within each particle and the interparticle voids. Often, an increase in solid density is accompanied by a decrease in apparent densities as porosity develops during pyrolysis. The density of the biochars depends upon the nature of the starting material and the pyrolysis process (Pandolfo et al. 1994).

According to the reports of Byrne (1996), Kercher and Nagle (2002), and Downie et al. (2012) solid density of biochar increases with the increase in process temperature and longer heating residence time. As the gases devolatilize from the solid biomass structure during pyrolysis, they leave a porous char behind. This porous structure left behind directly influences the number of other properties, such as water-holding capacity, mechanical stability, and cation-exchange capacity. It has been found that increasing pyrolysis temperature leads to higher porosities of the char (Weber and Quicker 2018). The higher the porosity, the lighter the char per unit volume. Moreover, lower amounts of volatiles, which have lower molecular weights than fixed C and lower ash contents, result in higher solid density in biochars (Jankowska et al. 1991). However, Brown et al. (2006) showed that density is independent of heating rate, and found a simple and direct dependency of density upon final pyrolysis temperature (Downie et al. 2012).

5.1.2 Surface Area of Biochar

It has been found that the surface area of the biochar produced from wood increases with increasing pyrolysis temperature to some extent after an initial increase, and the surface area of biochars may decrease again at much high temperatures. The reason behind this decrease is likely the result of a shrinking solid matrix (Quicker et al. 2016). Moreover, an increase in residence time leads to a further increase in the surface area, but even a very long residence time of many hours is less efficient in raising the surface area in comparison to an abrupt increase in temperature (Wang et al. 2012).

5.1.3 Pore Volume and Pore Size Distribution

Pores in biochar span several orders of magnitude and can be classified into macropores (with a pore diameter of 1000–0.05 μm), mesopores (0.05–0.002 μm), and micropores (0.05–0.0001 μm) (Brewer et al. 2014). As must be expected from the increasing porosity, the total pore volume increases with temperature (Angin 2013; Fu et al. 2012). The pore structure of biochars consists to a large extent of micropores, which may account for more than 80% of the total pore volume (chars from safflower seed cake, temperatures between 400 and 600 °C) (Angin 2013). As a comparison, the amount of micropores in untreated agricultural residues (straw and stalk) was determined to be less than 10% of the total pore volume of the biochar (Fu et al. 2012).

5.1.4 Water-Holding Capacity and Hydrophobicity

Biochar is known to have a water-repellent nature or hydrophobicity. And it has been found that hydrophobicity increases with increasing pyrolysis temperature (Pimchuai et al. 2010; Chun et al. 2004). The reason of this nature of biochar is the removal of polar functional groups present on the surface and also the increase in aromaticity during the process of pyrolysis (Cantrell et al.; Fang et al. 2014; Al-Wabel et al. 2013; Chun et al. 2004). Contradictory to this some authors have shown that higher temperature treatment may again reduce the hydrophobic character. According to the reports of Zornoza et al. (2016) biochars produced from crop residues and pig manure at 300 °C were classified as hydrophobic, but did not exhibit any hydrophobicity when treated with temperature above 500 °C. Similar results were made by Kinney et al. (2012). On this basis authors characterized chars into less and extremely hydrophobic biochars. Biochars produced at temperature more than 500 °C are less hydrophobic and low-temperature chars are extremely hydrophobic. The hydrophobicity of biochars is associated with remaining aliphatic functional groups. These aliphatic groups are destroyed at temperatures between 400 °C and 500 °C (Gray et al. 2014; Zornoza et al. 2016; Kinney et al. 2012; Das et al. 2015). All these features explain why torrefaction (typically up to 300 °C) converts a hydrophilic biomass into a hydrophobic char, but further increase in temperature to more than 500 °C may result in the loss of this hydrophobicity. It is important to note that the biochar does not become hydrophilic at higher temperatures, because no polar functional groups are formed. It merely becomes less hydrophobic due to the absence of nonpolar functionalities.

In addition, the effect of increasing porosity at higher temperatures becomes more dominant and the char may absorb more water. The water-holding capacity, the ability of a material to contain and retain water, depends on the porosity and interconnectedness of pores. Biochars produced at high temperature can therefore be expected to hold more water in their porous structure. This has been confirmed by experiments performed by Gray et al. (2013) and Zhang et al. (2013).

5.1.5 Mechanical Stability

The mechanical stability generally correlates directly with density and inversely with the porosity of the biochar. Any process that leaves cracks in the solid structure will result in a lower strength of the biochar. This mainly includes the release of volatile matter and water evaporation. Therefore, a low moisture content of the feedstock and a slow heating rate are beneficial for the production of biochar with high mechanical strength (Kumar et al. 2010; Noumi et al. 2014; Weber and Quicker et al. 2018).

5.2 Chemical Properties of Biochar

The composition of biochar is an important indicator for the determination of applications of biochar. This section focuses on the chemical properties of biochars. It reviews proximate and elemental composition, energy content, pH values, reactivity, degradation, and self-ignition processes that occur during storage in dependence on the process condition temperature and residence time.

5.2.1 Atomic Ratios

The process of carbonization or pyrolysis involves the changes in the chemical structure of the fuel, mostly by detachment of functional groups. This applies to both naturally occurring bio- and geochemical carbonization as well as technical carbonization for biochar production. The release of these hydrogen- and oxygen-containing groups results in a decrease in the respective ratios with carbon. The progression of the carbonization process can therefore be described by the evolution of the atomic ratios. Van-Krevelen diagram was given for technically produced biochar, both for woody feedstocks and for herbaceous and strawlike biomasses. The rise in treatment temperature leads to a decrease in both H/C and O/C ratios (van Krevelen 1950).

5.2.2 Elemental Composition

It was found that an increase in the reaction temperature leads to an increase in carbon content while resulting in a lower content of hydrogen and oxygen. The relative composition of biochars based on dry and ash-free matter is given in this section. The carbon content of untreated woods is typically slightly above 50% and the oxygen content just above 40% (by weight, dry ash-free) (Vassilev et al. 2010). The most significant changes during biochar production occur in the temperature range of 200–400 °C. High-temperature biochars may reach carbon contents of more than 95% and oxygen contents of less than 5%. The hydrogen content of wood varies between 5% and 7% and is decreased during pyrolysis to less than 2% (for temperatures above 700 °C) or even below 1% for very high treatment temperatures. Carbon, oxygen, and hydrogen contents of woody biochars evolve very homogeneously with rising temperature despite the different species and process conditions considered. A slight increase in the content of nitrogen was also found with increasing temperature. This relative increase is due to the decrease in other components during the devolatilization process (Quicker et al. 2016).

5.2.3 Energy Content

As a result of the higher carbon content in the biochar, the energy content of biochar increases with temperature. Heat treatment at 700 °C leads to an increase in energy content from 15 to 20 MJ/kg for raw biomass to 30–35 MJ/kg for biochar (Ibbenbüren et al. 2008). The most significant increase in energy content takes place at temperatures between 250 °C and 350 °C. Within this range of only 100 °C, the heating value is raised from less than 20 MJ/kg to values of 25–30 MJ/kg. Beyond 400 °C, the change in energy content is not significant. Prolonging the residence time also has a positive effect on the heating value, leading to a further increase. However, the effect is rather small compared to that of the temperature (Pach et al. 2009).

5.2.4 Fixed Carbon and Volatile Matter

The carbon content that remains in the solid structure after the volatile components are driven off is referred to as fixed carbon. Some biochar applications, especially metallurgical, require very high fixed carbon contents of more than 90% or even 95% in order to substitute fossil carbon carriers (Quicker et al. 2016). The fixed carbon content of raw biomass is in the range of 10–30% and undergoes no significant change before the torrefaction range. Between 250 °C and 350 °C, the amount of fixed carbon is increased to about 50–60%. Even though this small temperature range shows to have the most effect on the fixed carbon content, for the formation of fixed carbon contents of more than 90% temperature of about 700 °C is required. Moreover, the content of volatile matter is inversely related to fixed carbon content.

5.2.5 Presence of Functional Groups

The main process during carbonization is the thermal decomposition of the biomass structure, resulting in the detachment of functional groups and the release of oxygen and hydrogen. As a result, biochars with low H/C ratios (corresponding to a higher degree of carbonization) contain less functional groups and more aromatic structures than low-temperature chars (Conti et al. 2014; Rutherford et al. 2004). Aromatic structures have a high thermodynamic stability and are therefore important for some applications such as soil amendment or metallurgical purposes, where long-term stability of biochar is required.

5.2.6 pH of Biochar

The pH value of biochars increased with temperature, probably as a consequence of the relative concentration of non-pyrolyzed inorganic elements, already present in the original feedstocks (Novak et al. 2009).

5.2.7 Cation-Exchange Capacity

The cation-exchange capacity (CEC) is the amount of exchangeable cations (e.g., Ca^{2+}, Mg^{2+}, K^+, Na^+, $NH4^+$) (Leeq et al. 2010) that a material is capable of holding. It is a result of negative surface charges attracting cations and is used to describe the fertility of soils because almost all nutrients used by plants and microbes are taken up in their ionic form (Robertson et al. 1999). The cation-exchange capacity therefore directly depends on the surface structure, with functional groups providing surface charges, and the surface area, making the surface charges accessible (Liang et al. 2006). In order to measure the amount of exchangeable ions, the material (i.e., soil or char) has to be brought into a solution. The results of the measurement strongly depend on the pH value to which this solution is prepared (Helling et al. 1964), for example by using different solvents such as distilled water, NaOH, or HCl. A higher pH value will result in a higher cation-exchange capacity (Leeq et al. 2010; Mukherjee et al. 2011).

6 Biological Properties of Biochar

Biochar is a natural, unique, stable soil enhancer which is rich in carbon, porous with fine grains and produced as a result of incomplete vegetation fires under limited oxygen supply and historic management practices. A variety of vegetation (called as feedstock) can be used for biochar preparation including wood chips, bark, crop residues, food industry waste, animal manure, sewage sludge, microalgae biomass, etc. Soil amendments with biochar make soil fertile by changing its physiochemical and biological properties of soil in addition to carbon sequestration of soil (Sohi et al. 2010; Ibrahim et al. 2013; Krishnakumar et al. 2013; Hosseini et al. 2015). When compared to its source, biochar is both chemically and biologically more stable. Characteristically, the properties of biochar differ mainly depending upon its source; however, key features of biochar include its adsorptive property that mainly alters the surface area, pore size distribution (micro- and macropores), bulk density, water-holding capacity, and penetration resistance of the soil to which it is amended (Oshunsanya and Aliku 2016).

Soil biota forms an integral part of ecosystem by breaking organic matter, nutrient cycling, and soil formation. The biological properties of biochar help in protecting these microbes from unfavorable conditions by providing microbial habitat and refugia for them. This has been seen when normal soil was compared with biochar-added soil; the amended soil promotes higher microbial biomass with lower microbial activity (Thies and Rillig 2009; Oshunsanya and Aliku 2016; Gluszek et al. 2017; Elliston and Oliver 2019). Alternately, in comparison to non-oxidized biochar, when the oxygen content increases and carbon content decreases in biochar particles, even the microorganisms change the properties of the biochar (Cheng et al. 2008). Attributed mainly to its absorbing properties, biochar is also known for alleviating factors that inhibit soil microbes; hence it increases soil nitrification activity such as nitrification potential, net nitrification, and gross nitrification and modifies nitrogenase dynamics in soil too which are usually absent in grassland soils (DeLuca et al. 2006; Gluszek et al. 2017).

Predatory soil micro/mesofauna such as protozoans, nematodes, mites, or collembola which cause predation are also controlled by biochar, thereby acting as a refuge for soil microorganisms (Thies and Rillig 2009). Since it increases nutrient and water availability, it may minimize the abundance of mycorrhizal fungi by suppressing mycorrhizal symbiosis requirements (Warnock et al. 2010) but favors *Pythium* or *Phytophthora* (zoospore-forming pathogens) in comparison with bulk soil (Thies and Rilling 2009). Under high salinity, biochar incorporation shows a positive response on arbuscular mycorrhizal fungi and *Aeromonas hydrophila*, *A. caviae*, and *Bacillus insolitus* (plant growth-promoting rhizobacteria, PGPR) (Jaafar 2014; Yu et al. 2019) that consists of ACC (1-aminocyclopropane-1-carboxylate deaminase) (Ali et al. 2014). Additionally, bacteria and fungi have extracellular enzymes that degrade substrates including C derived from biochar into smaller molecules and are taken up and used for metabolic activities (Dahlawi et al. 2018).

Presence/absence of moisture and nature of biochar too influence the soil meso- and microfauna (Gluszek et al. 2017) as in case of earthworms (*Eisenia fetida*) w.r.t. dry biochar they prefer wetted biochar as it affects their mortality and causes weight loss too (Liesch et al. 2010; Li et al. 2011) but reduces the adverse effects of organic pollutants and hence positively increases their growth and reproduction (Denyes et al. 2012; Malińska et al. 2016). This might be because earthworms might develop some physiological mechanisms such as antioxidant defense (Sanchez-Hernandez et al. 2019). However, depending on the nature of nutrients adsorbed on the surface of biochar, different microorganism communities either flourish or are suppressed (Muhammad et al. 2014; Cui et al. 2016).

Table 1 Effect of biochar amendments in mitigating heavy metal toxicity in plants

Sr. no.	Plant species	Heavy metals	Biochar type	Biochar doses	Observed effect	Heavy metal removal rate	References
1.	Rice	Cd	Wheat straw at 350–550 °C	0, 10, 20, and 40 t ha^{-1} after wheat harvest	Increased soil pH by 0.15–0.33 units and 0.24–0.38 units	Cd reduced by 32.0, 39.2, and 52.5% and by 5.3, 43.4, and 39.8%, rice grain Cd uptake reduced by 16.8, 37.1, and 45.0% and by 14.4, 35.9, and 45.9%	Cui et al. (2011)
2.	Wheat	Cd	Wheat straw at 350–550 °C	0, 10, 20, and 40 t ha^{-1} at a depth of 0–15 cm during rice season	Increased soil organic carbon, increased soil pH by 0.11–0.24 and 0.09–0.24 units	Cd reduced by 10.1–40.2% and by 10.0–57.0%; total wheat Cd uptake decreased by 16.8–37.3% and by 6.5–28.3%	Cui et al. (2012)
3.	Wheat	Cd, Zn, Pb, As	Rice residues (straw, husk, bran) biochar	5% ratio based on dry weight	Increased shoot biomass (21%), root length (70%), shoot P level (59%), shoot K level (40%)	Decreased shoot Cd (71%), Zn (37%), Pb (60%) but increased As (199%)	Zheng et al. (2013)
4.	Barley, beans	As, Cu, Zn, Cd, Ni	Wood-derived (*Fraxinus excelsior* L., *Fagus sylvatica* L., *Quercus robur* L.) biochar pyrolyzed at 450 °C, 48 h	0, 25, 50 t ha^{-1}	Increased pH and EC especially at higher conc.	Small differences in total metal concentrations observed among treatments for As, Cu, and Ni but not for Pb and Zn	Lucchini et al. (2014)

(continued)

Table 1 (continued)

Sr. no.	Plant species	Heavy metals	Biochar type	Biochar doses	Observed effect	Heavy metal removal rate	References
5.	Maize plants (*Zea mays* L.)	Fe, Mn, Zn, Cd, Cu, and Pb	*Conocarpus* biochar	0.0, 1.0, 3.0, and 5.0% (w/w) and two soil moisture levels (75% and 100% of field capacity, FC)	Decreased bulk density, increased soil moisture content, increased shoot dry biomass	Reduced shoot heavy metal conc. to increasing application rates, highest decrease Mn—51.3%; 60.5% Zn—28%; 21.2% Cu—60%; 29.5% Cd—53.2%; 47.2% At soil moisture levels of 75% FC and 100%	Al-Wabel et al. (2015)
6.		Cd, Pb, Zn	*Miscanthus sinensis* Andersson biochar		Increased soil pH	Reduced Cd and Zn	Houben and Sonnet (2015)
7.	Jack bean (*Canavalia ensiformis*), *Mucuna aterrima*	Cd, Pb, and Zn	Sugar cane straw-derived biochar produced at 700 °C	1.5%, 3.0%, 5.0% (w/w)	No differences in the internal structures of leaves	Decreased available concentrations of: Cd—56% Pb—50% Zn—54%	Puga et al. (2015)
8.	*Spinacea oleracea* L.	Ni (0, 25, 50 and 100 mg Ni/Kg soil)	Cotton stick-derived biochar	0%, 3%, 5%	Increase in growth, photosynthetic, physiological, biochemical traits	Decreased Ni conc. in root and shoot at 3–5%	Younis et al. (2015)
9.		Zn		30% (by volume)	Showed soil leachate conc.	Little change in total Zn	Peltz and Harley (2016)

Sr. no.	Plant species	Heavy metals	Biochar type	Biochar doses	Observed effect	Heavy metal removal rate	References
10.	Wheat	Cd	Rice straw	0%, 1.5%, 3.0%, and 5% (w/w)	Increased pH, silicon content, decreased bioavailable Cd, increased plant height, spike length, shoot and root dry mass, grain yield, antioxidant enzymes	Cd decreased by 26%, 42%, and 57% in wheat grains	Abbas et al. (2017)
11.	*Brassica juncea*	Zn, Pb, Cd, and Cu	Bamboo biochar	0%, 1%, 2.5%, 5%	Improved shoot/root growth, dry biomass, enhanced chlorophyll (*a* and *b*), carotenoid conc., accelerated enzymatic activities (β-glucosidase, alkaline phosphatase, and urease)	Reduced heavy metal uptake in the shoot/root	Ali et al. (2017)
12.	*Lycopersicon esculentum*	Ni, Mn, Cr in serpentine soil	*Gliricidia sepium* (Jacq.) biomass and woody biochar BC300 (produced at 300 °C) BC500 (produced at 500 °C)	0, 22, 55, 110 t ha^{-1} (0, 1.0, 2.5, 5.0% by mass)	Improved plant growth, decreased metal toxicity; C, N, S contents increased, reduced heavy metal bioavailability (BC500-110 most effective for metal immobilization)	Reduction in: Ni—19–30% Cr—1–50% Mn—58–60%	Bandara et al. (2017)
13.		Cd-, Cu-, Pb-, and Zn-contaminated mine land soils	Feedstocks (pine beetle-killed lodgepole pine; *Pinus contorta*) and tamarisk (*Tamarix* spp.)	0, 5, 10, and 15% by wt.	Increases in soil pH (initial, 3.97; final, 7.49) and 55–100%	Decreased bioavailability of: Cd—55–100% Cu—91–100% Pb—84–100% Zn—60–100%	Ippolito et al. (2017)

(continued)

Table 1 (continued)

Sr. no.	Plant species	Heavy metals	Biochar type	Biochar doses	Observed effect	Heavy metal removal rate	References
14.	Dwarf beans (*Phaseolus vulgaris* L.)	As, Sb, Pb	Commercial charcoal obtained from wood biomass (500 °C)	2%, 5%	Increased soil and soil pore water (SPW) pH, EC and soil water content (SWC)	Decreased labile Pb conc., increased As and Sb solubility	Lomaglio et al. (2017)
15.		Contaminated soil surrounding copper smelter	Bamboo and rice straw biochars	0, 1, and 5% (w/w)		Lowered conc. of Cd, Cu, Pb, and Zn (especially at 5% application rate)	Lu et al. (2017)
16.	Brachiaria (*Brachiaria decumbens*)	Al, Mn, Zn, Fe, Cu	Oil palm crop residues	0, 1, 2.5, 5 g	Enhanced the germination and root length, Munsell color, texture, pH, electrical conductivity, water-holding capacity, cation-exchange capacity, metal content, organic carbon, sulfates, extractable P, total nitrogen		Muegue et al. (2017)
17.	*Cassia alata* L.	Multi-metal Mine tailings	*Hibiscus cannabinus* core (HB)	0.4% (w/w)	Root biomass, shoot biomass, plant height and root length increased	Decrease in: Pb—63.9–89.5% Zn—46.9–66.0% Cu—32.7–62.4% Cd—40.4–76.4% As—54.9–77.5%	Huang et al. (2018)
18.		Cd-, Ni-, Zn-contaminated soil	Modified coconut shell biochar (MCSB)	0%, 2.5%, and 5% addition for 63 days	Higher soil biological activity, maximum bacterial number increased by 149.43% at 5% MCSB	Cd—30.1% Ni—57.2% Zn—12.7% Decrease at 5% amendment	Liu et al. (2018)

Sr. no.	Plant species	Heavy metals	Biochar type	Biochar doses	Observed effect	Heavy metal removal rate	References
19.		Pb–Zn-contaminated soil	Co pyrolysis of rice straw with swine manure at 400 °C	R:M ratios: 1:0 3:1 1:1 1:3 0:1	Increased yield, ash content, soil pH, electrical conductivity, and dissolved organic carbon (DOC) concentrations	Reduced metal conc. in order of Pb > Cu > Zn > Cd	Meng et al. (2018)
20.		Pb	Peanut hull	0, 2.5, 5.0, and 10 g kg^{-1} soil by weight for 55 days	Increased soil phosphorus content, no significant differences in pH and organic matter content	Reduced Pb conc.	Sabijon and Poliquit (2018)
21.	Wheat	Cd, Pb	Wheat straw biochar	0, 20, and 40 t ha^{-1}	Increased grain yield	Reduced Cd, Pb conc. in wheat grains	Sui et al. (2018)
22.	Moso bamboo (*Phyllostachy pubescens*)	Cu	Wood biochar, bamboo biochar, rice straw biochar, and Chinese walnut shell biochar	5%	Increase in plant biomass, dry weight, improved soil electrical conductivity, reduced solubility of soil heavy metals	Reduced Cu uptake; bamboo biochar—15% Straw biochar—35% Chinese walnut shell biochar—26%	Wang et al. (2019)
23.	Paddy soil	Cd	Wheat straw pyrolyzed at ~450 °C	0, 5, and 15% biochar as wt:wt or wt:vol in soil and solution resp.	Promoted formation of (oxy) hydroxide, carbonate, organically bound Cd phases; elevated pH (~1–4 pH units)	Removal of up to ~90% from Cd-containing solutions and contaminated soil	Cui et al. (2019)
24.	Cress (*Lepidium sativum*)		Corncob biochar	10, 20, and 30 t/ha	Germination rate, shoot length, fresh shoot weight, decreased with increased biochar conc.		Intani et al. (2019)

(continued)

Table 1 (continued)

Sr. no.	Plant species	Heavy metals	Biochar type	Biochar doses	Observed effect	Heavy metal removal rate	References
25.		Zn (II) and Cd (II)	Wheat straw (WS) biochars			Ions in porewater increase Q_{Zn}/Q_{Cd} in alkaline soils but reduce in acidic soils	Qian et al. (2019)
26.	Chinese cabbage	Pb, Cu, Zn, Cd	Straw block biochar: maize straw biochar; peanut shell biochar: maize straw biochar	Mass ratio of 1:2 (SDM); mass ratio of 2:1 (DSM); mass ratio of 2:1 (DPS)	Increased pH, EC, available P and K of soil	Decreased Pb and Cu concentration in soil; decreased Pb, Zn, and Cu conc. in plants	Xu et al. (2019)

7 Application of Biochar for Mitigation of Various Heavy Metal Phytotoxicity

With increasing anthropogenic activities, biochars may play a role as a safe, unharmed means for reducing bioavailable concentrations of heavy metals/metalloids both in water bodies and soils which may lead to accumulation in food chain which might cause irreversible changes and deterioration of human health. Therefore, numerous studies have been and are being carried out where biochars from a variety of feedstock have been employed especially as soil amendment to minimize the devastating effects of heavy metals and their bioavailability on varied plant species. Studies on application of biochar for mitigation of various heavy metal phytotoxicity have been summarized in Table 1.

8 Conclusion

Biochar applications offer economic feasibility and environmental sustainability solutions. One of the most important biochar properties is large surface area and cation-exchange capacity which facilitate sorption of organic as well as inorganic contaminants, and thus possibly help in reduction of various organic and inorganic pollutants as well as heavy metal mobility. However, biochar is one of the most cost-efficient and environmentally friendly solutions for mitigation and remediation of heavy metal contaminants. The functions of biochar properties totally depend upon the type of feedstock used and production technology. Thus selectivity of suitable biochar is crucial and needs more attention.

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Cellular and Molecular Phytotoxicity of Lead and Mercury

Aryadeep Roychoudhury and Swarnavo Chakraborty

Contents

1 Introduction

Heavy metal contamination is considered as a global environmental issue. Increased industrialisation and urbanisation have resulted in rise in concentrations of various heavy metals in the biosphere (Cheng 2003; Yabe et al. 2010; Azevedo and Rodriguez 2012), thus imposing adverse effects on plant metabolism and growth (Nagajyoti et al. 2010). Post-transition elements like lead and mercury are two of the major heavy metals that have been found to be potential environmental pollutants. Contamination of the environment with these metals occurs due to increased mining and smelting activities, use of paints, pesticides and explosives, disposal of contaminated sewage and combustion of fuels (Chaney and Ryan 1994). Toxic metals like lead and mercury can generate reactive oxygen species (ROS) in plant systems (Gratao et al. 2005) by interference with the electron transport chain. Increased levels of ROS in plant cells induce oxidative stress, finally leading to peroxidation of membrane lipids generating pores in the membrane, DNA damages, degradation

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of various biological macromolecules and disruption of ion channels (Sergio et al. 2000; Rakhshaee et al. 2009).

Mercury compounds can exist either in elemental state (Hg^0) or in +1 or +2 oxidation states, while lead compounds exist in +2 or +4 oxidation states. However, for both lead and mercury, +2 oxidation states are more stable and occur more commonly in nature. It is found that plants do not take up either lead or mercury on purpose, as none of them are essential for plants, but plants growing in contaminated soils and waters demonstrate higher uptake rates (Kumar et al. 2017). Plants can not only absorb mercury via their roots, but can also absorb mercury deposited on the surfaces of leaves. However, aquatic plants rather demonstrate more mercury uptake as compared to terrestrial plants. Interestingly, not only plants absorb lead from soil or deposit them on leaf surfaces, but lead can also enter tissues through broken surfaces. The toxic effects of lead and mercury on plants have posed serious threats to agriculture due to sharp fall in crop productivity (Johnson and Eaton 1980). Mercury and lead pollutants have become almost indispensable in this era of modern life and contamination due to these heavy metals being unlikely to reduce in the near future (Yang et al. 2000). Therefore, mechanisms of phytoremediation and detoxification of these toxic metals like phytoextraction and rhizofiltration have come up these days, to reduce the critical risks of heavy metal toxicity on agriculture, human health and the environment. This review focuses on the modes of uptake and transport of lead and mercury, their adverse effects on plant metabolism and possible ways of remediation and tolerance in plants to nullify the ill effects of lead and mercury contamination.

2 Sources of Lead and Mercury

Lead and mercury are major pollutants in both aquatic and terrestrial ecosystems. Smelting and mining of ores, automobile exhausts, factory chimneys, use of fertilisers and pesticides, plating of metals, effluents of battery industries, urban soil wastes and use of additives in gasoline are the common sources of lead contamination in the environment (Sharma and Dubey 2005). Use of mercury in amalgamating gold and silver, upcoming chlor-alkali industries and production of cinnabar and various pigments have considerably increased the global release of mercury (Hylander and Meili 2003). Burning of coal, increased smelting and use of lime, manure and mercury-containing fertilisers and pesticides are also potential reasons for such sharp increase in the present times.

3 Transport and Distribution of Lead and Mercury in Plants

3.1 Lead Transport and Distribution

Plants take up lead from contaminated soils and water or in the form of aerosols. Lane and Martin (1977) demonstrated that significant quantities of lead can be taken up via the roots, but translocation to upward plant parts is very poor. However, it was later found in *Zea mays* L. that depending on the concentration, lead can be translocated to the above-ground parts and appreciable quantities of lead was found to be accumulated in the leaves (Miller and Koeppe 1971). Lead deposited in the form of aerosols on the surfaces of leaves can also be absorbed aerially, but the rate of absorption depends on the morphology of the leaves. For instance, downy leaves demonstrate higher aerial absorption of lead. The availability of lead to the roots depends on various soil factors like pH of soil and size of individual soil particles. Usually with the increase in pH of the soil, the absorption of lead via roots also increases, but within a pH range of 5.5–7.5, the availability of lead to the roots is restricted due to precipitation in the form of carbonates and phosphates (Blaylock et al. 1997). Covalent association in organic forms like binding of lead to carboxyl groups of uronic acids on root surfaces and colloidal precipitation of lead can equally restrict its availability to the roots. Plant factors like the rate of transpiration, root surface area, release of exudates from roots and symbiotic associations like mycorrhiza can also modulate the uptake of lead through roots. Post-uptake by the roots, lead tends to get accumulated in the roots due to interaction with carboxylate of glucuronic acid and galacturonic acid in the plant cell walls, thus limiting transport of lead via the apoplastic route. However, this was more commonly demonstrated in dicots as compared to the monocots (Huang and Cunnigham 1996).

Almost no information is available regarding the presence of specific lead transporters in plants, as this toxic metal is not essential for plants. The voltage-gated cation channels, for example, calcium channels present in the plasma membrane of root cells, participate in lead uptake (Huang et al. 1994). Plants growing in toxic concentrations of lead displayed rather lower calcium transport via these calcium channels, as lead was competitively transported into cells, by preventing the normal uptake of calcium. The casparian strips of endodermis act as a barrier to lead transport by apoplast, thus maximising lead transport through the symplast to the above-ground plant parts. With the increase in distance from the roots, the concentration of lead in aerial parts of the plants reduced drastically, with the lowest concentration in young leaves (Godzik 1993). Seeds with unruptured testa did not accumulate lead as compared to seeds with disrupted testa due to growing radicle. Exceptionally meristematic zones of developing radical and hypocotyl of the developing seed showed negligible uptake of lead. To counteract the toxic effects of lead, various plant species were found to develop pinocytic vacuoles due to plasmalemmal invagination (Ksiazek et al. 1984). These vacuoles can sequester these excess metal ions and tend to occupy an extensive volume of the cell wall near the plasmodesmata.

Similarly, Samardakiewicz and Wonzy (2000) found formation of numerous vacuoles inside the cells of the aquatic plant *Lemna minor* L. grown in lead-contaminated waters. Therefore terrestrial as well as aquatic plants combat the toxic effects of lead via sequestration of lead in vacuoles.

3.2 Mercury Transport and Distribution

Similar to lead, mercury also tends to accumulate in the roots and translocation to aerial parts like leaves, flowers and developing organs is limited (Sierra et al. 2009). However, Esteban et al. (2008) reported the presence of a separate transporter system for mercury uptake in white lupin plants, but in case of most plants, iron, copper and zinc transporters usually bring about the influx of mercury by competitively inhibiting these metals into root cells. So, the levels of essential metals like copper, zinc and iron get severely depleted in plants growing in mercury-contaminated soils. Despite its potential toxic effects, the presence of mercury transporters in white lupin was really surprising, and the answer to this is still a mystery. The absorption of mercury by the roots increased appreciably in the presence of high concentrations of arsenate in soils (Du et al. 2005). This might be due to increase in negative charge of the cell wall by arsenate, thereby up-regulating mercury uptake. Restriction of mercury uptake via roots due to precipitation in the form of insoluble phosphate, sulphate and carbonate, leading to accumulation of mercury in root cell walls, was noted similar to lead transport. Apart from roots, leaves also play a major role in mercury influx and accumulation, from mercury deposited on leaf surfaces in the form of aerosols emitted by industries (Fay and Gustin 2007). The question regarding how leaves take up mercury from air is still unresolved; probably, the stomata are responsible for this uptake during the process of gaseous exchange. However, depending on the morphology of leaves and the type of plant species, this uptake of mercury can vary considerably. Soil factors like pH, particle size, cation-exchange capacity and aeration also modulate the rates of mercury uptake (McGrath et al. 2001).

4 Phytotoxicity Induced by Lead and Mercury

4.1 Lead-Induced Phytotoxicity

Entry of lead inside plant cells brings about an array of negative effects on various physiological aspects of the plants. Stunted growth, reduction in growth of roots, chlorosis, deficiency of essential minerals, disruption of hormonal and enzymatic activities and membrane status, and water shortage are the common symptoms of lead toxicity in plants. Verma and Dubey (2003) demonstrated decreased germination rate and fall in normal growth of rice seeds with increased lead in growth media.

Initially upon lead exposure, the lateral root branching patterns get altered, with considerable decrease in primary root length, probably due to inhibition of cell division in the root cells by competitively lowering calcium uptake, during lead toxicity (Eun et al. 2000). Upon excess lead treatment in onion cells, various irregularities in mitosis were observed, with nuclei having irregularities in shape and disrupted nuclear content, changes in the microtubular alignment and spindle formation (Wierzbicka 1994; Yang et al. 2000). Therefore, lead inhibits root cell division by altering the alignment of microtubules. Apart from inhibition of cell division, lead was also found to disturb the ion channels on the plasma membrane of root cells. For instance, in corn seedlings, potassium ion leakage from root cells increased with the rise in lead levels in media (Malkowski et al. 2002). Lead toxicity leads to drastic fall in overall dry weight of plants and reduction in DNA, RNA, protein and lipid contents, thereby reducing permeability of the chloroplasts. Changes in levels of monogalactosyl diacylglycerols in membranes of lead-affected plants altered the permeabilities of chloroplast membranes. The fluidity of the cell membranes changed due to increase in unsaturation and reduction in saturation of membrane fatty acids with increased lead exposure (Stefanov et al. 1995).

Lead can exert an inhibition in the activities of various enzymes, thereby altering a range of metabolic pathways in plants. This inhibition in activity is due to binding of lead to the –SH groups of cysteine in enzymes and formation of mercaptide, so that the overall tertiary structure of the enzymes gets disrupted and the enzymes cannot function optimally. Moreover, interaction of lead with the –COOH and phosphate groups present in the enzymes can even downregulate the enzyme activity. Lead has the power of replacing other metal ions from the active site of metalloenzymes, thus disrupting their function. Essential plant enzymes like δ-aminolaevulinate dehydrogenase involved in the biosynthesis of chlorophyll (Prassad and Prassad 1987), nitrate reductase (Paivoke 2002), pentose phosphate pathway (PPP) enzymes (Hampp et al. 1973), α-amylase, ribulose bisphosphate carboxylase/oxygenase (Vallee and Ulmer 1972), glutamate dehydrogenase (Lee et al. 1976) and ATP synthetase (Tu Shu and Brouillette 1987) were all severely inhibited by increased lead concentrations in soils. However, no considerable effects of lead toxicity were seen on DNase and RNase, and malate and lactate dehydrogenase, activities. Interestingly, pyruvate kinase activities were upregulated due to increase in lead nitrate levels in soils (Vallee and Ulmer 1972), probably due to destruction or cross-linking of inhibitors of this enzyme by lead. Hydrolytic enzymes like peroxidases and some acid phosphatases and few proteases increased in plants growing in lead-contaminated soils, leading to leaf senescence and even plant death (Lee et al. 1976). Lead-mediated oxidative stress in plants, due to rise in reactive oxygen species (ROS), caused up-regulation of antioxidative enzymes like glutathione reductase, ascorbate peroxidase, guaiacol peroxidase and superoxide dismutase (Verma and Dubey 2003), but the levels of antioxidative metalloenzymes reduced to a great extent. *Ceratophyllum demersum* plants grown in water containing excess of lead salt displayed major changes in the chloroplast ultrastructure (Rebechini and Hanzely 1974), due to disruption of lipid constitution of the membranes in thylakoids (Stefanov et al. 1995). Even the activity of enzymes involved in Calvin cycle is also lowered due to lead toxicity, thereby affecting the rates of photosynthesis in plants. Moreover, restrain in the biosynthesis of carotenoids, plastoquinone and chlorophyll; disrupted chloroplast structure due to fall in the number of grana stacks, stroma and starch grains; and obstructed electron transport chain due to lead toxicity also decreased the photosynthetic and respiratory rates to a great extent. Lead toxicity in plants can severely affect the respiratory rate and the ATP levels in plant cells by uncoupling of the oxidative phosphorylation. However, in mitochondria of leaves detached from some C_3 plants like pea (Romanowska et al. 2002), higher rates of oxidation of glycine, malate, etc. were observed followed by increased ATP/ADP ratio, with increasing lead concentrations in the nutrient solution. The mechanisms associated with this respiratory upregulation still remain undeciphered. Most astonishingly, the oxygenase activity of the photosynthetic enzyme, ribulose-bisphosphate carboxylase/oxygenase, remains almost unaltered due to lead toxicity, unlike the carboxylase activities (van Assche and Clijsters 1990). This implies that photorespiration will continue, but photosynthesis will be severely disrupted in the affected plants. Due to the fall in rates of photosynthesis, the ATP/ADP ratio rises inside plant cells. Lead-treated plants displayed a higher ATP/ADP ratio, irrespective of inactivation of ATP synthetase by excess lead. This probably occurs due to lesser utilisation of ATP for carbon dioxide fixation, as photosynthesis is greatly disrupted in these plants. Burzynski (1987) deciphered that uptake of elements like magnesium and iron by plants was competitively reduced by lead, thus affecting chlorophyll synthesis in plants. The involvement of two photosystems, PSI and PSII in the light reaction of photosynthesis, is a well-known fact. Amongst the two, PSII is rather more affected due to rise in intracellular levels of lead (Sersen et al. 1998). Lead causes retention of several ions in the roots of plants. Therefore, lead toxicity reduces the uptake and distribution of elements like potassium, calcium, magnesium, nitrogen, phosphorus and iron (Walker et al. 1977) and sometimes manganese and sulphur. Plants growing in lead-contaminated soils show disturbed nitrogen metabolism with lower nitrate reductase activities (Paivoke 2002). Therefore, affected plants have higher root nitrogen levels and lower nitrate concentrations due to inhibition of nitrate reductase activity.

One of the most prominent effects of lead toxicity in plants is the upregulation of oxidative metabolic pathways. Large amounts of reactive oxygen species (ROS) are generated as a result of oxidative stress, and they include hydrogen peroxide (H_2O_2), hydroxyl radical (OH), singlet oxygen ($^1O_2$) and superoxide anion (O_2^-) (Das and Roychoudhury 2014). Depending on the span and propensity of stress and the physiology of the plant, the generation of ROS due to lead toxicity varies considerably (Verma and Dubey 2003). During oxidative stress in plants, the primary symptoms include decrease in the content of saturated fatty acids in the membrane and increase in the generation of lipid peroxides due to peroxidation of membrane fatty acids (Roychoudhury et al. 2012a), and lead is found to rather enhance these adverse processes. Verma and Dubey (2003) found the upregulation of a number of enzymatic and non-enzymatic antioxidants in response to lead-induced oxidative stress in plants, in order to combat the ill effects of ROS. The commonly up-regulated non-enzymatic antioxidants include tocopherol, ascorbate, carotenoids, glutathione, etc., while the enzymes include peroxidases, ascorbate peroxidase, catalase, superoxide dismutase, NADPH-dependent glutathione reductase, dehydroascorbate reductase, monodehydroascorbate reductase, etc. (Roychoudhury and Basu 2012). The respective genes involved in the biosynthetic pathways of these antioxidants were modulated by stress, induced due to increased lead concentrations inside cells, thereby leading to changes in expression patterns of these molecules in stressed plants.

Exogenous application of lead on plants caused triggering of nitric oxide (NO) signalling cascades inside plant cells. Lead-treated plants displayed higher rates of production of NO, which acts as a key signalling molecule in diverse types of biotic and abiotic stress in plants. Tewari et al. (2008) explained the antioxidative role of NO in scavenging the harmful ROS species, produced as a result of stress in affected plants. Two key enzymes, nitrate reductase (NR) and nitric oxide synthase (NOS), are predicted to be associated with NO generation in plants. However, concrete evidences to support these facts are still not available.

4.2 Mercury-Induced Phytotoxicity

Ge et al. (2009) demonstrated the stunting in growth of wheat plants with increased mercury concentrations in the media. Later, other groups also demonstrated the same in other plant species establishing that plants exposed to mercury toxicity displayed a shortening of height (Zhou et al. 2008) due to several changes in cellular ultrastructure like changes in cell shape and size, reduction in intercellular spaces and disruption of vasculature (Chen et al. 2009). One of the key enzymes involved in the biosynthesis of chlorophyll, NADPH-dependent protochlorophyllide oxidoreductase, was found to be downregulated in stressed plants, thus reducing chlorophyll content by disruption of thylakoids in leaves (Lenti et al. 2002). Mercury can competitively reduce the transport and distribution of other elements like magnesium, and can replace magnesium from chlorophyll molecules, thus severely reducing photosynthetic rates (Patra et al. 2004; Cargnelutti et al. 2006). Like most heavy metal-induced stress, mercury stress also induces the production of ROS in plants. The earliest ROS to be generated upon stress inception due to mercury is superoxide anion (O_2^-), which may trigger the production of other forms of ROS like hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen ($^1O_2$). These ROS lead to oxidative damages in plants like disrupting proteins and peroxidation of lipids on membranes and serve as common biomarkers for stress due to toxicity in plants (Meng et al. 2011).

An array of genes in pea including *PsSMAT, PsPOD, PsAPSR, PsNDA,* etc. were found to be actively expressed in stressed plants. These are involved in the biosynthesis of isoflavonoids and antioxidants, salicylic acid-mediated defence pathways and maintenance of rigidity of cell wall. A range of genes involved in chlorophyll biosynthesis and secondary metabolite production were also found to be modulated due to mercury toxicity (Heidenreich et al. 2001). The commonly upregulated antioxidants include non-protein thiols and glutathione, along with a range of antioxidant enzymes like ascorbate peroxidase, superoxide dismutase, NADPH-dependent glutathione reductase, NADH oxidase, catalase and peroxidase (Sparks 2005). Mercury has the tendency to bind to –SH groups of cysteine residues and phosphate groups in proteins and disrupt protein tertiary structure and functioning. Increased mercury concentrations inside plant cells can reduce sugar, nitrogen, DNA and RNA contents, thus severely affecting the growth of plants.

Interestingly, mercury can induce mutations by covalently associating with DNA (Patra et al. 2004). Mercury, even in low dosage, can provoke chromosomal aberrations, exchanges of sister chromatids, c-mitosis and disruption of spindle arrangement. Ribose sugars, purine and pyrimidine present potential interaction sites like N and O, for mercury to interact with DNA. Recently, the elevation in the levels of few signalling molecules like nitric oxide (NO) and carbon monoxide (CO) has been reported in plants exposed to increased mercury levels (Hsu and Kao 2004; Guo et al. 2009). These display a protective role against oxidative damages and increase the levels of tolerance against mercury-induced stress in plants. NO can potentially reduce the generation of H_2O_2 in plants and reduce the severity of oxidative damages. Proline, an important osmo-protectant in plants, is also upregulated in mercury-stressed plants (Zhang et al. 2008), which was proved by the rise in levels of proline biosynthesis enzyme, Δ^1-pyrroline-5-carboxylate synthetase (P5CS). Usually the two enzymes, nitric oxide synthase (NOS) and nitrate reductase (NR), are associated with NO generation in plants. Irrespective of detection of considerably high amounts of NOS activity in stressed plants, the *NOS* gene is still unidentified in plants (Gas et al. 2009). NO can also reduce the translocation of detrimental heavy metals and thus can reduce the levels of toxicity in plants. On the other hand, CO is generated by haem oxygenase (HO) enzyme and this remains almost conserved in a wide range of plant species. Some research groups have reported the existence of four genes, *HO-1, HO-2, HO-3* and *HO-4*, in *Arabidopsis* plants, amongst which *HO-1* is the most studied and plays a pivotal role in CO-mediated signalling. CO can enhance tolerance levels in plants by reducing the levels of ROS generated as a result of mercury-induced oxidative stress.

5 Tolerance Against Lead and Mercury

Baker (1981) explained two basic strategies depicting the metal uptake and their tolerance limits in plants. They are “excluder” and “accumulator” strategies. The former one involves the maintenance of an almost constant low concentration of a particular heavy metal until a critical concentration is attained, which is followed by excessive metal uptake leading to toxicity, while the latter one depicts an extraordinary capability to accumulate a particular heavy metal within plant tissues at varying concentrations of the metal, to escape toxic effects. Later, It was postulated that plants can evade the detrimental effects of heavy metals by either biochemical tolerance or avoidance or detoxification of the metal involved. This led to the identification of various tolerant plant varieties, with high production of oxalate ions. Oxalate helps to reduce lead uptake via roots by precipitating lead and restricting its transport (Yang et al. 2000). Cell wall carbohydrates and uronic acids present carboxyl groups that can bind lead and prevent metal translocation. Lead-tolerant varieties of *Anthoxanthum odoratum* play an active role to restrict lead uptake and distribution through their cell wall (Quereshi et al. 1986). Mercury hyper-accumulator *Sesbania drummondii* displayed much higher expressions of antioxidant enzymes like ascorbate peroxidase, catalase, superoxide dismutase and peroxidase as compared to the sensitive plants (Israr et al. 2006). Development of pinocytic vesicles and sequestration of lead in vacuoles are commonly observed in leaves of tolerant plants treated with lead salt solution.

Hyper-accumulators of lead and mercury display higher levels of amino acids like proline, polyamines like putrescine and metal-chelating polypeptides like phytochelatin, which is characterised by ϒ-Glu-Cys dipeptide repeats followed by a terminal glycine (Zenk 1996). Phytochelatins can bind a range of heavy metals with varying affinities, and lead to sequestration of these metals, thus causing heavy metal detoxification. Hyper-accumulator and tolerant varieties thus have higher expressions of phytochelatin synthase for greater rate of phytochelatin biosynthesis. Tolerant plants develop more callose and suberin depositions in their cell wall and increased production of stress-related hormones like abscisic acid and ethylene (Seregin and Ivaniov 2001). The amino acid proline is found to play an immense role in heavy metal stress; for instance, proline serves as an osmolyte, chelates metals, stabilises protein structure, scavenges ROS and inhibits peroxidation of lipids (Roychoudhury et al. 2015). An important thiol compound glutathione has also been proved to bind heavy metal ions and prevent their uptake. With the increase in the severity of stress, the levels of glutathione in plants have been shown to increase proportionately. It acts as a redox-buffering system and combats the harmful consequences of ROS in cells. Glutathione in cells can exist in two different forms, the oxidised form of glutathione (GSSG) and the reduced form (GSH). Both these forms always maintain equilibrium and the ratio gets altered upon the inception of stress (Garrido et al. 2010). In different plants, glutathione can act as an important signalling molecule, maintains the redox homeostasis inside the cells and mediates the process of xenobiotic detoxification. Glutathione (GSH) is generated as a tripeptide, and contains three amino acids, namely glutamic acid (Glu), cysteine (Cys) and glycine (Gly). GSH is commonly referred to as ϒ-glutamyl-cysteinyl-glycine (Mullineaux and Rausch 2005). The oxidised form of glutathione (GSSG) is generated in a redox cycle, by the establishment of a disulphide linkage between two molecules of the reduced form of glutathione, i.e. GSH, coupled to the reduction and oxidation cycle of NADPH and a proton (H^+). The two protons liberated as a result of generation of GSSG are utilised in the ascorbate-glutathione cycle and this cycle is responsible for the elimination of the harmful ROS species. Another class of polypeptides with high cysteine content and lacking histidines and aromatic amino acids is the metallothioneins. They are composed of a conserved Cys X Cys sequence, X depicting any amino acid except cysteine (Lu et al. 2003). The metallothioneins can bind heavy metals under toxic concentrations via the thiol group (−SH group) of the cysteine residues, sequestering the undesired toxic metals and protecting the plants from adverse effects.

6 Phytoremediation of Lead and Mercury

The term phytoremediation refers to the role of plants in mediating the uptake of inorganic and organic contaminants, to clean up the environment and improve the quality of environment. Phytostabilisation, phytovolatilisation, rhizofiltration and phytoextraction are commonly used strategies for phytoremediation of inorganic pollutants, while for organic pollutants, phytostabilisation, phytodegradation, phytovolatilisation, phytodegradation and rhizofiltration are exclusively used (Roychoudhury et al. 2012b).

Phytoextraction strategies involve the accumulation of mainly inorganic contaminants in shoots and other aerial parts of the plant, which can be later harvested (Kumar et al. 1995), thus removing the contaminant from the soil and making it suitable for other plantations. Several plants like *Zea mays* and *Brassica juncea* have been reported to act as hyper-accumulators of toxic metals and are exploited to revive contaminated soils. Rhizofiltration defines the power of plant roots to absorb or adsorb certain pollutants, mainly metals like lead and other organic or inorganic pollutants from contaminated soils and water bodies (Dushenkov et al. 1995). The metals are either absorbed and accumulated in the roots or precipitated, to clean up the contamination, without the manifestation of any morphological changes in the plants that carry out the process. This precipitation of inorganic contaminants is referred to as phytostabilisation; this occurs due to interaction of pollutants with root exudates secreted by certain plant species. Burken and Schnoor (1999) explained the removal of volatile contaminants like mercury from soils by the action of tolerant plants and named this process as phytovolatilisation. The role of the *merA* gene, which is an altered form of mercuric reductase gene, and the *merB* gene, encoding the organo-mercurial lyase, was deciphered, and transgenic plants were developed. Transgenic *Arabidopsis* with cloned *merB* (organo-mercurial lyase) or *merA* (mercuric reductase) gene displayed the power of volatilising mercury into the atmosphere. These enzymes carry out the biological conversion of methyl mercury by organo-mercurial lyase to Hg (II), followed by conversion to a volatile form of mercury (Hg^0), by the enzyme mercuric reductase using an electron donor, NADPH. Phytodegradation and rhizodegradation involve the role of plant roots and symbiotically associated rhizospheric microbes to clean up the contaminated soils. Microorganisms mainly remediate soil contaminated with organic pollutants (Burken and Schnoor 1997). Presently, these environment-friendly and cost-effective technologies have come up and have provided potential solutions to remediate the contaminated environment and combat the adverse effects of such pollutants on life.

However, there are a number of limitations for the phytoremediation technology. The method is quite time consuming and is dependent on the prevailing climate and soil conditions. Also, the age and physiology of the plant to be used for the phytoremediation purpose do play an important role (Tu et al. 2004). Younger plants with high metabolic rates are more efficient phytoremediators than old plants of similar size. The depth of roots and the surface area, and the volume of roots, act as limiting factors.

The greater the spread of roots into the soil, the greater the extent of accessibility of roots to the contaminants, and hence the faster the rate of phytoremediation. The contaminants should come in direct contact with the roots. Several cycles of harvesting of the accumulator plants have to be carried out and disposed as potential hazardous wastes as per proper rules and regulations. Failing to follow the requisite regulations, other food chains will get contaminated (Mwegoha 2008).

7 Conclusion and Future Perspectives

Lead and mercury toxicity in plants has gained great attention in this present era, due to increased load of anthropogenic activities in the environment. Sharp fall in crop growth and yield is commonly manifested in plants exposed to increased lead and mercury. Both these toxic metals are taken up via plant roots, with minute concentrations absorbed by the aerial parts. Post-entry into cells, these metals modulate the activities of various enzymes and hormones, trigger various signalling cascades, disrupt cellular homeostasis due to disruption of mineral and water balance, change membrane integrity and induce oxidative stress by ROS generation. Both cellular and molecular changes are brought about by the adverse effects of these metals. Genome-wide transcriptome analysis helped scientists to determine the genes that were induced due to toxic metal stress and thus decipher probable insights of transcriptional and post-transcriptional regulatory networks in plants. This has helped them to understand and predict probable roles of these genes and their modes of regulation upon accumulation of heavy metals in plants. Biochemical tolerance against lead and mercury in plants is associated with the changes in cell wall constituents, upregulation of antioxidants and other enzymes and amino acids. The extent of alteration of these parameters in response to the toxic metals serves as the biomarker indicating the degree of tolerance of different plants to different metal concentrations. Genetic and biochemical insights of tolerant and hyper-accumulator plants aided by biotechnological tools will provide probable solutions to generate plants with increased tolerance limits. Genetic manipulation of plants and induced molecular breeding can act as important strategies to generate tolerant varieties, demonstrating higher levels of mercury and lead tolerance and reducing the risks of toxicity in animals and humans feeding on these crops. Phytoremediation and detoxification programmes are carried out using several plant species to clean up contaminated soils and waters. However, this demands further studies and research, to effectively exploit these techniques for the sake of conservation of the environment, as well as identify the plant species and their mechanistic regulation in cleaning up the pollutants.

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Cadmium Toxicity and Its Mechanism in Plants

Sonu Kumari, Monika Yadav, and Suphiya Khan

Contents

1 Introduction

Recently the environmental pollution due to different nonessential heavy metals have become an alarming situation over the globe (Anjum et al. 2016). Heavy metals enter into the agricultural ecosystem and transfer to human beings through food chain (Fig. 1). The soil acts as the major source of transference of heavy metals to plants and animals in terrestrial ecosystem. The heavy metal enters into the plant systems, leading to various consequences, and affects the crop productivity as well as grain qualities. Heavy metals having specific density and atomic mass 40 comprise a large number of elements. Most of these metals have marked high affinity towards sulfur-containing ligands. Therefore, when such heavy metals enter the cell, they efficiently interact with SH group and disturb many metabolic processes by inactivating many enzymes.

Of all the nonessential heavy metals, cadmium (Cd) metal has been considered as an important pollutant in soil science and plant nutrition due to its high toxicity to human beings and the relative mobility in the soil plant system. The Cd pollutants of the environment resulting from various industrial activities exhaust gases of automobile, agricultural, and mining activities (Foy et al. 1978). The potential concerns for Cd transference and accumulation in the food chain were highlighted by Schroeder and Balassa (1963). The most magnificent symptoms of Cd toxicity

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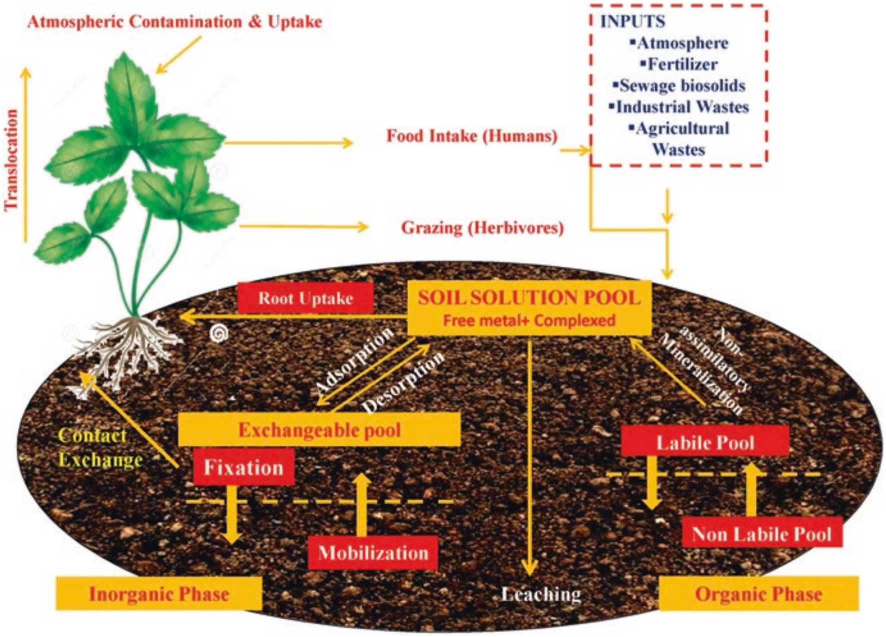

Fig. 1 Cd movement in soil, plant, and food

on plants are red-brown coloration of leaf margins or veins, growth retardation, necrosis, and chlorosis of leaves. Cd disturbs water balance, mineral nutrition, photosynthesis, respiration, and growth rate of plants (Prasad 1995).

Plants have different mechanisms to restrict or deal with the overindulgence of heavy metals in soils. The stress avoidance mechanism helps to prevent the metal uptake by plants or reduce the toxic effects of heavy metal/ions inside the protoplast via stress tolerance (Siedlecka et al. 2001). The stress avoidance mechanisms of plants in the aspect of Cd stress are rather of insignificant relevance. Stress tolerance mechanisms are much more important and Cd ion forms complex with organic acids, amino acids, and cysteine-rich peptides (Prasad 1995).

2 Cd Sources and Effect on Humans

Cd heavy metal is present in soil and water as a lethal metal which is available in oxidation state as 0 or +2. It stays in environment as cadmium sulfate, cadmium hydroxide, and cadmium carbonate. Different precipitated forms present are chromates, arsenates, sulfides, and phosphates. According to USEPA, the Cd permissible limit for soil is less than 1 mg L^{-1} (Table 1) (Mahajan and Kaushal 2018). The two chief causes of Cd are environmental and human activities (Satarug et al.

Table 1 Permissible limit given for Cd

Standards	Permissible limit (mg L^{-1})
IS10500	0.003
WHO	0.003
USEPA	0.005
EU	0.005
NHMRC, Australia	0.002

2011). Therefore, the Cd contamination in environment occurs either due to straight manufacturing or by secondary sources.

The main sources of Cd for humans are water and consumption of vegetables; its high amount of consumption causes multiple malfunctioning of organs (Ryan et al. 1982). Its high accumulation causes liver damage and Itai-Itai disease (Fig. 2). Its anticipated average half-life period in human body and ecosystem is about 10 and 18 years, respectively (Forstner and Wittmann 2012; Salt et al. 1995). In plants, it affects photosynthetic machinery, plant growth, leaf chlorosis, and necrosis, and inhibits oxidative reactions and nitrogen metabolism (Foy et al. 1978).

3 Cadmium Toxicity in Soil

In soil solution the complex of metals is based on the pH of soil and the availability of other metals (Das et al. 1997). It is identified that normal soils have Cd concentration up to 0.32 mM (Wagner 1993). Thus soil having Cd content range from 0.32 to 1 mM is classified as moderate-level contaminated soil (Sanita de Toppi and Gabbrielli 1999). Toxicity may result due to the attachment of metals to sulfhydryl of protein that leads to reduced enzyme activity and damage of protein structure. Inhibition of enzyme activity after interaction to metals is because of covering of catalytically active groups (Das et al. 1997).

4 Cadmium Uptake and Translocation Mechanism

The bioavailability of metals is restricted due to lower mixing in oxygenated water and efficient attachment to soil grains. Cd accessibility depends mainly on soil concentration, temperature, redox potential, pH, and content of various metals. Cd is considered as a hazardous metal because of its enhanced movement in soil and lower amount found to be toxic to plants. The Cd ion uptake by plants is reported as in the struggle for identical transmembrane transporter with nutrients like Ca, Mg, Fe, K, Mn, Cu, Zn, Mn, and Ni (Rivetta et al. 1997). Cd is effectively transported inside plants as metallorganic complexes (Epstein and Bloom 2007). Rhizosphere

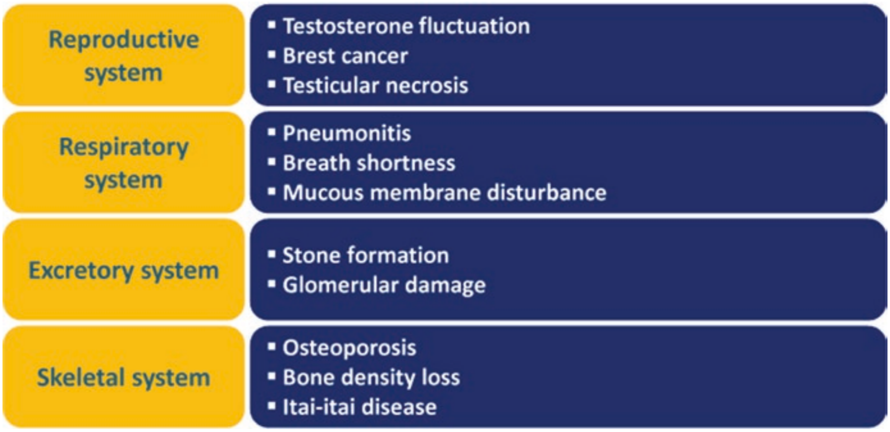

Fig. 2 Impact of Cd on various systems of human body

acidification and carboxylase exudation are considered as possible targets for upgrading accretion of metals (Clemens et al. 2002). Cd initially enters the plant roots and initially influences the root route (Sanita de Toppi and Gabbrielli 1999). The electrochemical potential gradients between the cytosol and the root apoplasts control the absorption of Cd across the plasma membrane of root cells. To drive the Cd^{2+} uptake even at low doses, a strong driving force is given by the membranes (Hirsch et al. 1998). The apoplast of the root epidermis and cortex is highly permeable for solutes. As Cd enters the roots, the apoplastic or a symplastic pathway facilitates its transmission to xylem (Salt and Dushenkov 1995). There are following three mechanisms that manage the transference of metals from the root:

- Segregation within root
- Symplastic transfer in stele
- Discharge in xylem (Clemens et al. 2002)

After reaching xylem Cd forms complex by interacting with several ligands like organic acids and phytochelatins (Senden et al. 1994). Usually Cd ions reside in roots and just less concentrations are moved to the shoots. The Cd content in plants reduces in the arrangement roots > stems > leaves > fruits (Blum 1997) and this statement is favored by Moral et al. (1994) by reporting Cd ion absence in the developing fruit of tomato.

Due to heavy metal treatment, plant demonstrates antioxidant system in mainly chloroplasts and mitochondria where respiratory and photosynthetic electron transfer chains work. These enzymes work for catalysis of reactions or function in direct processing of ROS. Enzymatic and nonenzymatic antioxidant defense processes are present as GOPX, MDHAR, CAT, GST, DHAR, APX, SOD, GR, alkaloids, α-tocopherols, GSH, nonprotein amino acids, and AsA. Competition of metals with other mineral nutrients with same properties is dependent on the mechanism of Cd uptake into plants through root system. Mineral reduction present in plants as Mg, K, and Ca is reported with high uptake of Cd in cucumber, maize, lettuce, and tomato plants. Inverse relation was observed between Zn and Cd with their absorption sites in root tissues of lettuce. However, some elements like nitrate which is not similar to Cd are easily affected by Cd presence. The Cd uptake through root cells is controlled by the cytosol and root apoplasts' electrochemical potential. Required energy for Cd movement at low concentration is provided by the large membrane potential. Energy of Cd uptake by roots displays biophasic qualities with saturable contents at lower Cd functions in the absorption mixture and linear components at greater Cd functions (Gill and Tuteja 2010). Uptake of Cd can similarly occur as inorganic compounds or organic complexes as phytometallophore complexes.

Cd is a toxic element that is proved harmful for both plant growth and development because of its higher movement in soil-root-shoot scheme (Vázquez et al. 2006). After the entering of Cd in root membranes through apoplast, various nutrient pathways as Ca, Zn, and Fe are accountable for the adsorption of Cd in the root system of plants (Cosio et al. 2004; Nakanishi et al. 2006). Highest accumulation is observed in the root tissues and a small amount is translocated in other portions of the plant (Kovacik et al. 2006; Gill et al. 2012). Its uptake and accumulation are mostly based on soil properties and varieties of plants (Arao et al. 2003). It was reported that Cd uptake was 7–12 times higher in the roots of *Matricaria chamomilla* than leaves (Kovacik et al. 2006). However, in *Crotalaria juncea* the Cd accumulation in leaves was only 6% of root at a concentration of 2 mM $CdCl_2$ (Pereira et al. 2002). Jiang and his coworkers reported that *Allium sativum* accumulated 1826 times more Cd in roots of contaminated plants as compared to standard under 10^{-2} M treatment (Jiang et al. 2001). Higher presence of Cd was obtained in the root tissues as compared to shoot in rice plants (Shah and Dubey 1997). Cell wall is the main accumulation site for green microalgae such as *Eremosphaera viridis, Chlorella vulgaris*, and *Ankistrodesmus braunii* (Hosayama et al. 1994). Researchers identified that root tissues act as an obstruction for Cd absorption and movement in rice plants. Also, the amount of Cd differs between plant species (Grant et al. 2008). Considerable difference was observed in Cd amount of *Triticum aestivum, Linum usitatissimum,* and *Helianthus annuus* (Li et al. 1997). Cataldo et al. (1983) observed that only 2% Cd is present in shoot tissues and rest 98% was present in root tissues of *Glycine max* plants. About four- to fivefold Cd accumulation in root tissues of *Solanum lycopersicum* plants was reported as compared to mature leaves (Djebali et al. 2008). Metal movement from soil to root tissues of plant and further movement to other plant parts are responsible for toxicity of metal (Verma and Dubey 2003). Several factors are accountable for the presence of Cd and other metals as bioavailability of metal and different elements' ratio in soil (Brümmer et al. 1986). High accumulation is reported in cell vacuole and a small amount is found in Golgi apparatus, nuclei, chloroplast, and mitochondria (Malik et al. 1992a, b).

Accumulation of metals is dependent on the carboxylase exudation and acidification of rhizosphere. Cd and Zn are reported to be co-present in the aboveground area of *Arabidopsis* plants (Bert et al. 2003). Report suggests that Cd and Zn movement is genetically linked and either these are transported by one transporter or their transporters are managed by same regulators. Molecular techniques facilitate the identification of many cation transporters. Majority of transporter proteins considered of being concerned in the transfer of micronutrients fall under ZIP (ZRT, IRT-like protein) and Nramp (natural resistance-associated macrophage protein) family (Williams et al. 2000) (Fig. 3). In developing seeds Cd might connect to phytate in globoid crystals inside the protein structure. Different metals like Ca, Fe, Zn, and Mn have also been accounted for to be coupled with phytate inside globoid crystals.

5 Cadmium Toxicology Effects in Plants

Cadmium being an unnecessary component is harmful for plant development rate. It has been identified as an important and noteworthy toxin because of its elevated toxicity and rapid solubility in water (Pinto et al. 2004). Cadmium can interfere the transfer of minerals in plants through disturbing the presence of minerals in the soil, or by reducing the inhabitants of soil microbes (Moreno et al. 1999). Its presence in nutrient solutions affects the stomatal opening, photosynthesis, and transpiration rate in plants. Chlorosis, wilting, leaf curl, and plant growth retardation are the chief and simply noticeable signs of Cd harm on plants. Chlorosis could emerge to be iron shortage, phosphorus absence, or decreased Mn uptake (Goldbol and Hutterman 1985). Cd stress inhibits root Fe (III) reductase that is responsible for Fe (II) shortage and affects photosynthesis (Alcantara et al. 1994). It also reduces the uptake of nitrate and transference from roots to aerial parts, through inhibiting the function of nitrate reductase in aerial parts (Hernandez et al. 1996). During Cd treatment nitrogen fixation and primary ammonia assimilation are found to be reduced in nodules

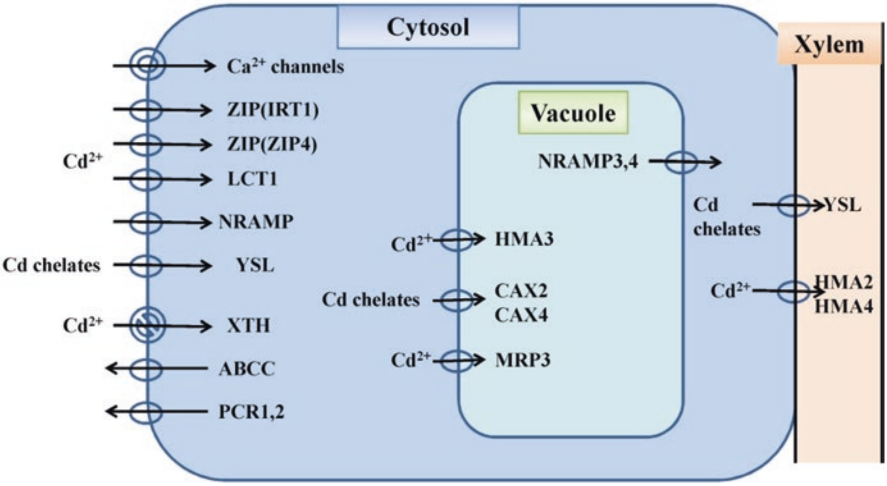

Fig. 3 Cd invasion to the symplasm, sequestration in vacuoles of root cells, and efflux to rhizosphere and xylem

of soybean plant (Balestrasse et al. 2003). Its toxicity alters the membrane porosity and results in a decline in water concentration (Costa and Morel 1994). Cd presence is reported to decrease ATPase action of membrane fraction isolated from wheat and sunflower roots (Fodor et al. 1995). Cd treatment manifests modifications in the activity of plasma membranes and turbulence in chloroplast metabolism through reducing chlorophyll production. Soybean plants incorporated with Cd are recognized under oxidative stress (Balestrasse et al. 2001).

Several biochemical, structural, and physiological pathways are severely affected by the Cd presence in plant structure (Khan et al. 2007, 2009). Cd present in cell inhibits stomatal opening, reduces crop productivity, and disturbs nutrient uptake and photosynthesis (Table 2). It indirectly affects different mechanisms and thus induces the oxidative stress. As soon as Cd reaches and accumulates in plant body, it activates the function of NADPH oxidases that concludes in lipid oxidative burst, and SOD and H_2O_2 buildup (Brahim et al. 2010). It also enhances the phytochelatin synthase activity which results in high production of phytochelatins. As phytochelatins are oligomers of reduced glutathione (GSH), this results in reduction of the GSH pool, a significant metabolite in upholding the cellular redox balance.

Alanine aminotransferase and hexokinase are the two essential enzymes which are induced at 10 μM Cd treatments and reported to be responsible for cellular result to different abiotic stresses (De Sousa and Sodek 2003). These enzymes are proved essential for glutamate synthesis which forms glutamylcysteine and GSH. Accessible knowledge on harmful results of different physiological mechanisms of plant results in research on the introduction of Cd-resistant function in plants. Cd severally affects membrane function through its attachment with protein sulfhydryl group of enzymes. Function of H^+-ATPase, contributing to the movement of metals through roots, in cell membrane of roots of papilionaceous plants is reported to be consider-

Table 2 Cd effects on plants' different parameters

Parameters	Effects	References
Root system	Reduction of root length, enhanced cortex cell volume, epidermis affected	Kidd et al. (2001); Vazquez et al. (1992)
Cellular concentrations	Unbalanced cellular content of micronutrients	Hernandez et al. (1998); Zhang et al. (2000)
Photosynthetic system	Photosynthesis damage; stomatal opening damaged in *Syzygium aromaticum*, *Glycine max*, and *Medicago sativa*; chlorosis; chlorophyll a and b concentration affected; reduced photosynthetic rate; photosystems I and II disturbed	Barcelo et al. (1986); Viehweger and Geipel (2010); Siedlecka and Baszynsky (1993)
Protein	Protein synthesis reduction	Tamas et al. (1997)
Lipid peroxidation	Membrane breaking, lipid composition abnormal	Hernandez and Cooke (1997); Stefanov et al. (1995)
Fresh and dry weight	Reduced fresh weight of *Vigna radiate,* decreased root and shoot weight of *Vigna ambacensis*	Wool (1983); Rana and Ahmad (2002)
Carbonic anhydrase	Affects the function of carbonic anhydrase	Siedlecka and Krupa (1996)

ably less than in the Cd-tolerant cucurbitaceous plants (Obata et al. 1996). Cd also breaks the DNA strands, DNA oxidative damage, and chromosomal aberrations which results in increased proliferation and altered DNA repair (Mouron et al. 2004).

In *Vicia faba*, Cd is proved to be both geno- and cytotoxic which leads to an indicator of DNA damage that is sister chromatid exchange (SCE). Both antioxidant enzymes and antioxidants work for protecting the plant from genotoxic effects of Cd and modifications (Table 3). Both apoptotic and intracellular signaling pathways are severely affected by Cd contamination (Ünyayar et al. 2010). Cd is also known to cause internode shortening by 40% and stem thinning with partial bleaching on leaves in *Elodea canadensis* plant (Vecchia et al. 2005). In *Pisum sativum* plants, root tissues are easily affected by Cd treatment as compared to shoot tissues (Metwally et al. 2005). It was obtained to inhibit root length, and restrain cell growth and leaf expansion (Zhou and Qiu 2005). Reduction in both leaf and root dry weight is reported in *Zea mays* on treatment with different Cd contents (Ekmekci et al. 2008). Ghnaya et al. (2005) obtained that it significantly inhibited *Mesembryanthemum crystallinum* development at a small amount. Kachout et al. (2009) reported that plant treatment with different concentrations of heavy metal (Cu, Ni, Pb, Zn) lowered the DM synthesis and shoot length in both *Atriplex hortensis* and *A. rosea* plants. Root tissues are more seriously affected by the harmful action of metals than shoot tissues. Root height of *Atriplex* was reduced to a great ratio when treated with 25%, 50%, 75%, and 100% heavy metal content for 4 weeks.

It was observed that seed germination of *Sorghum bicolor* plant was influenced when treated with 3 mM Cd treatment (Kuriakose and Prasad 2008). It was observed that necrosis of leaf tissues due to Cd treatment is the cause of Cd movement and its transfer to other plant parts above the ground. It was observed that when *Vigna radiate* was applied with Cd solution, it reduced the chlorophyll concentration, leaf dry weight, and its area. Reduction up to 40.0%, 30.8%, 39.8%, and 59.8% in chlorophyll content, photosynthesis, leaf surface area, and plant dry weight was obtained in Cd-treated PS 16 plants. For same parameters, decrement was observed up to 36.3%, 27.5%, 23%, and 26.2%, respectively (Anjum et al. 2011). It was reported for *Brassica juncea* plant that Cd treatment severely affected the chlorophyll, plant growth, and carotenoid content as compared to Pb. Reduction in protein amount at flowering phase was 95% at 900 μM Cd concentration and 44% at 1500 μM Cd

Table 3 Cd treatment modified antioxidant enzymes

Plant	Effects	References
Oryza sativa	CAT, SOD, APOX, GPOX, GR	Hsu and Kao (2004)
Saccharum officinarum	CAT, SOD, GR	Fornazier et al. (2002)
Arabidopsis thaliana	CAT, SOD, APOX, GPOX, GR	Cho and Seo (2005)
Helianthus annuus	CAT, SOD, APOX, DHAR, GR	Gallego et al. (1996)
Glycine max	CAT, SOD, APOX	Balestrasse et al. (2001)
Phragmites australis	CAT, SOD, APOX, GR	Iannelli et al. (2002)
Pisum sativum	CAT, SOD, APOX, GPOX	Dixit et al. (2001)
Triticum durum	CAT, SOD, APOX, GPOX	Milone et al. (2003)

content (Huang et al. 1974). With just a minute quantity of Cd in chl, various straight and indirect results are obtained, and the outcome is reduction of photosynthesis. Majority of scientists correlate the decline of chl in Cd-stressed plants with reduction of production.

6 Conclusion

Cadmium after mercury and lead is considered as the third major toxic contaminant. Cd being a nonessential heavy metal is found to be highly phytotoxic and has negative impact on plant growth. It retards various morphological, physiological, molecular, and biochemical activities of plants. Cd stress causes reduction in plant growth rate by affecting water or nutrient uptake, photosynthesis, and oxidative damage. The higher concentration of Cd in soil can cause death of the plant. Cd adversely affects the health of both humans and animals. Cd uptake from soil by plant root system and redistribution between root and shoot are facilitated by various metal transporters situated at the plasma membrane. This chapter highlights the effects of Cd on various plant activities by incorporating transference mechanism of metal from soil to plant parts.

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Toxicity of Tungsten Oxide and IAA-Loaded Tungsten Oxide Nanoparticles on *Linum usitatissimum* Germination and Their Antifungal Activity

Manikandan Madheslu, Narendhran Sadasivam, Simin Sabana Ameerbasha, and Vishnu Ramakrishnan

Contents

1 Introduction

Tungsten oxide nanoparticles gained more attention among metal oxide because of their surface and significant optical properties (Satyapaul and Madras 2013). The family of tungsten material have various technological applications like

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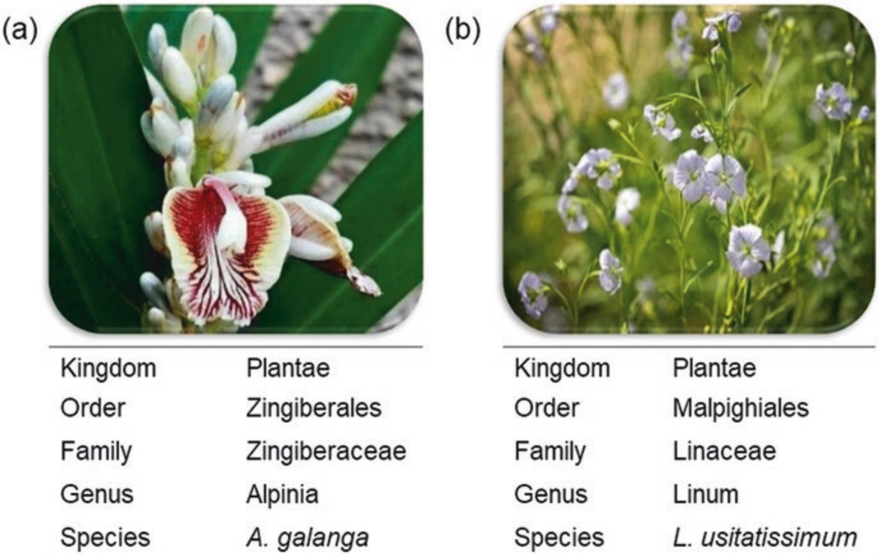
photocatalytic (Ashokkumar et al. 2013), optical modulation (Kulkarni et al. 2014), magnetic device (Zheng et al. 2011), gas sensing (Martinez-de-lacruz et al. 2010; Hariharan et al. 2011), antimicrobial (Ahmed et al. 2016) and anticancer activity (Tripathi et al. 2015) due to their band gap range from 2.0 to 3.5 eV. WO_3 NPs are synthesized by thermal decomposition, wet chemical process, pyrolysis, and hydrothermal methods (Abdel-Raouf et al. 2017; Sundrarajan et al. 2015). The antimicrobial activity of WO_3 NPs mainly depends on band gap energy, which is reduced by capping agent and indole-3-acetic acid used for coating metal oxide nanoparticles (Karakecili et al. 2019; Shukla et al. 2014). It has been proved as a plant growth-promoting nanoparticle, which has a complex structure with great optical properties (Lin and Xing 2008; Vreeland et al. 2016).

Alpinia galanga belongs to Zingiberaceae family and is used as a traditional medicine to cure inflammation, HIV, diabetes, and ulcer (Fig. 1a). It contains pharmacologically important metabolism such as galanolactone, quercetin, galangin, kaempferol, and labdane (Shukla et al. 2016). Banerjee et al. (2014) examined the antioxidant activity of leaf extract that contains phenolic group with the highest chelating and beta-carotene bleaching abilities (Kharissvoa and Jimenez Perez 2013). Due to their diverse pharmacological activity, *Alpinia galanga* leaf extract is used for the synthesis of WO_3 nanoparticles (Shukla et al. 2016).

Linum usitatissimum is an excellent source of nutrition and estrogen. It belongs to genus of Linum, which is commonly call as flaxseed (linseed) (Fig. 1b). Due to their health benefit and commercial use, it spread all over the world (Mane et al. 2014). Flaxseed has two types of varieties, such as brown and golden. Generally, flax plant grows up to 4 ft height having pale blue petals with slender stems



Kingdom	Plantae
Order	Zingiberales
Family	Zingiberaceae
Genus	Alpinia
Species	*A. galanga*

Kingdom	Plantae
Order	Malpighiales
Family	Linaceae
Genus	Linum
Species	*L. usitatissimum*

Fig. 1 Taxonomy of (**a**) *A. galanga* and (**b**) *L. usitatissimum*

(Renanathan et al. 2014). It contains about 40% oil content which is used as an active ingredient in bactericides, anticancer, antidiabetics, and disinfectants, because it contains natural antioxidant such as lignans. Mostly flaxseed is used in the food industry for oil. There is high demand for cultivation of plant in the domestic and international market. Usually flaxseed should be treated with antifungal agents, since it is highly susceptible to wilt diseases.

In the present investigation, we report the synthesis and characterization of nanoparticles (WO_3 and IAA-WO_3) using simple precipitation and characterized by UV-Vis, Fourier transfer infrared spectroscopy, X-ray diffraction, energy diffraction X-ray, scanning electron microscope, and dynamic light scattering analysis. To study the toxic level of synthesized nanoparticle by *Linum usitatissimum* seed germination using soaking method and antifungal activity against *A. niger*, *F. oxysporum*, and *P. funiculosum* was evaluated using well-diffusion method.

2 Materials and Methods

2.1 Materials

Alpinia galanga leaves were collected from Kanthalloor region (10.2135° N, 77.1972° E), Idukki district, Kerala, India. *Linum usitatissimum* (brown seed) was purchased from local market of Coimbatore. Plant fungal pathogens such as *Aspergillus niger* (MTCC: 10180), *Fusarium oxysporum* (MTCC: 3327), and *Penicillium funiculosum* (MTCC: 4888) were obtained from the Institute of Microbial Technology, Chandigarh, India. All chemicals were purchased from Sigma-Aldrich Chemicals, India.

2.2 Methods

2.2.1 Synthesis of WO_3 and IAA-WO_3 NPs

Tungsten oxide nanoparticles are synthesized according to the procedure suggested by Hariharan et al. (2011). This precipitation technique was used to synthesize tungsten oxide from sodium tungstate dihydrate ($Na_2WO_4 \bullet 2H_2O$, 99%) and *Alpinia galanga* leaf extract. 0.1 M of sodium tungstate was dissolved in a separate conical flask containing 100 mL deionized water (Subha et al. 2016). After vigorous stirring, 100 mL of plant extract (pH 3) was added as drops to this mixture and kept at 5 °C for 4 h, followed by precipitation for 1 day at room temperature. The precipitate was washed by adding deionized water followed by centrifuging at 5000 rpm for 5 min. After washing procedure, the precipitate was calcined at a temperature from 200 °C for 24 h. The obtained yellow color powder was stored in a screw-cap bottle for further use.

To synthesize IAA-WO_3 NPs, WO_3 NPs (0.1 mM) were mixed in a separate conical flask containing 50 mL deionized water. About 30 min of continuous stirring, 0.01 mg of indole-3-acetic acid was dissolved in ethanol, which was added dropwise and kept at 5 °C for 2 h. The precipitate was calcined at temperature from 100 °C for 8 h Karakecili et al. 2019; Kharissvoa and Jimenez Perez 2013; Hosseinpour-Mashkani and Nasab 2016). The obtained brown color powder was stored in a screw-cap bottle for further use.

2.2.2 Characterization of WO_3 and IAA-WO_3 NPs

The optical density and band gap energy are determined by UV-visible spectroscopy (V-650, JASCO). The crystalline structure of WO_3 and IAA-WO_3 nanoparticles is observed by X-ray diffraction with Cu Kα radiation (Perkin-Elmer spectrum). To study the functional group, which is responsible for the reduction and capping agent of nanoparticles, use Fourier transfer infrared spectroscopy (FTIR, Perkin-Elmer 1725×). Element composition and morphology of nanoparticles are analyzed using EDX (energy diffraction X-ray, Model QuanTax 200, Germany) and SEM (scanning electron microscope, Model JSM 6390LV). To determine the average size and distribution of nanoparticle, dynamic light scattering analysis is carried out (DLS, Malvern) (Hariharan et al. 2011; Kulkarni et al. 2014).

2.2.3 Assessing the Toxicity by *Linum usitatissimum* Germination

Linum usitatissimum seeds were immersed in a 2.5% sodium hypochlorite solution for 15 min for sterilization and experimental consistency was followed according to Lin and Xing (2008). After rinsing three times with Milli-Q water, they were soaked in WO_3 and IAA-WO_3 NP suspensions at various concentrations (0.1, 0.25, 0.5, 1, and 2 g/L) and at various intervals of 1, 2, and 3 days. Milli-Q water was used in the soaking process for a better sterility of media. A filter paper (Whatman No. 42, Maidstone, England) was placed in each Petri dish (90 mm × 15 mm); 5 mL of nanoparticle suspension was added in each Petri dish with 30 seeds. Petri dishes were sealed with parafilm and placed for incubation at room temperature. Following 7 days of treatment, seed germination was recorded by counting germinated seeds and rest of them were considered as non-germinated. Experiments were carried out in triplicate and mean values were recorded. Evaluate the conditions of seed germination index by relative germination rate and root elongation was calculated based on the following equations:

$$\text{Relative germination rate} = \frac{\text{Seeds germinated in test sample}}{\text{Seeds germinated in control}} \times 100$$

$$\text{Relative root elongation} = \frac{\text{Mean root length in test sample}}{\text{Mean root length in control}} \times 100$$

$$\text{Germination index} = \frac{\text{Relative germination rate}}{\text{Relative root elongation}} \times 100$$

2.2.4 Effect of WO_3 and IAA-WO_3 NPs on Biochemical Parameter of *Linum usitatissimum*

The biochemical parameters of WO_3 and IAA-WO_3 NP-treated *Linum usitatissimum* were assessed using standard procedures such as protein, total carbohydrates, and reducing sugar analysis.

2.2.5 Determination of Antifungal Activity of WO_3 and IAA-WO_3 NPs

Antifungal activities of the synthesized WO_3 and IAA-WO_3 NPs were assessed against plant fungal pathogen following well-diffusion method (Niraimathee et al. 2016). The pathogens were cultured in potato dextrose broth at room temperature on an orbital shaking incubator (Remi, India) at 200 rpm. A 100 μL of culture was swabbed on the potato dextrose agar (PDA) plates using sterile cotton swab. Then plates were allowed to stand for 10 min for culture absorption. The wells (5 mm size) were punched into the agar with the help of sterile gel puncher. Aliquots of 100 μL containing 50 and 100 mg/mL of the WO_3 and IAA-WO_3 NP solution and (10 mg/mL) positive control (amphotericin B) were poured into wells using a micropipette. The plates were incubated upside down at room temperature for 48 h. The zone of inhibition (diameter in millimeter) was measured and the mean values were recorded.

2.2.6 Statistical Analysis

All results are presented as mean ± standard deviation (SD). Using SPSS statistical tool, growth attribute was analyzed at the significant level ≤ 0.05 by T-test to test the difference between WO_3 and IAA-WO_3 NPs and control group. One-way analysis of variance (ANOVA) was also performed to test the effect of WO_3 and IAA-WO_3 NP dose on antifungal activity. *P*-values of ≤ 0.05 were considered statistically significant.

3 Results and Discussion

3.1 UV-Vis and FTIR Analysis of WO_3 and IAA-WO_3 NPs

Optical properties of WO_3 and IAA-WO_3 NPs were studied by UV-Vis spectroscopic analysis. The UV-Vis absorption spectrum of WO_3 and IAA-WO_3 NPs is shown in Fig. 2a, c in the range from 200 to 800 nm. To investigate band gap energy of synthesized nanoparticles utilize the optical density of UV spectral data using Tauc plot. To quantitatively derive the band gap of WO_3 and IAA-WO_3 NPs, formula $(Ah\nu/K)^2 = h\nu - E_g$ was employed in which A represents the absorption, K is the absorption constant, and E_g corresponds to band gap energy and slope of linear region to the x-axis interaction. Band gap energy of WO_3 and IAA-WO_3 NPs is shown in Fig. 2b, d and Table 1. The results show that the band gaps of WO_3 and IAA-WO_3 NPs are 2.0 eV and 2.82 eV, respectively.

The presence of functional group is determined by the FTIR spectrum in the range of 400–4000 cm^{-1} (Perkin-Elmer 1725×). Strongest peak around 600–800 cm^{-1} is responsible for O-W-O stretching vibration. The FTIR spectrum of *Alpinia galanga* aqueous extract and WO_3 and IAA-WO_3 NPs is shown in Fig. 3. The spectrum results indicate the presence of alkynes, carboxylic acid, and alcoholic and phenol function groups (Table 2). The peak observed in the region 3360 cm^{-1} is

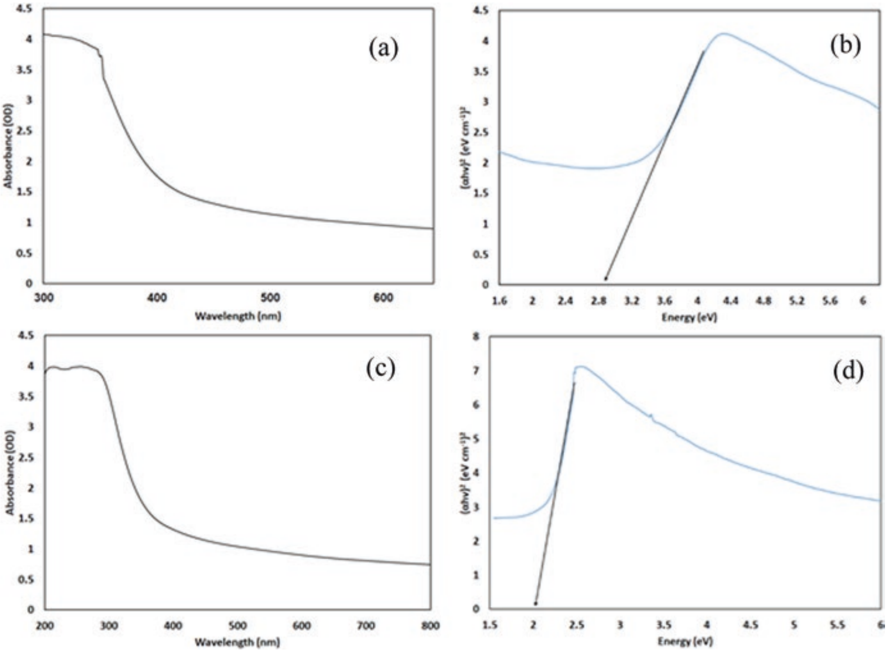


Fig. 2 UV-Vis absorption spectrum (**a**) (WO_3 and c—IAA-WO_3) and the plot of $(\alpha h\upsilon)^2$ versus ($h\upsilon$) of WO_3 (**b**) and IAA-WO_3 (**d**) NPs

Table 1 UV absorbance and band gap energy of WO_3 and IAA-WO_3 NPs

Nanoparticles	Absorption wavelength (nm)	Band gap energy (eV)
WO_3	368	2.82
IAA-WO_3	297	2.0

Table 2 FTIR analysis of *A. galanga* aqueous extract, and WO_3 and IAA-WO_3 NPs

Functional group and bond		Plant extract	WO_3	IAA-WO_3
Alkynes	C-Br stretch	690.22	692.44	686.99
Carboxylic acids	O–H bend	945.12	945.12	–
Primary amines	N–H bend	–	–	1622.13
Ester, alcohol	C-O (stretch)	1159.22	1159.22	–
Carboxylic acid	C=O stretch	–	1743.65	–
Alcohol and phenol	O-H stretch	3360.62	3398.57	3417.86

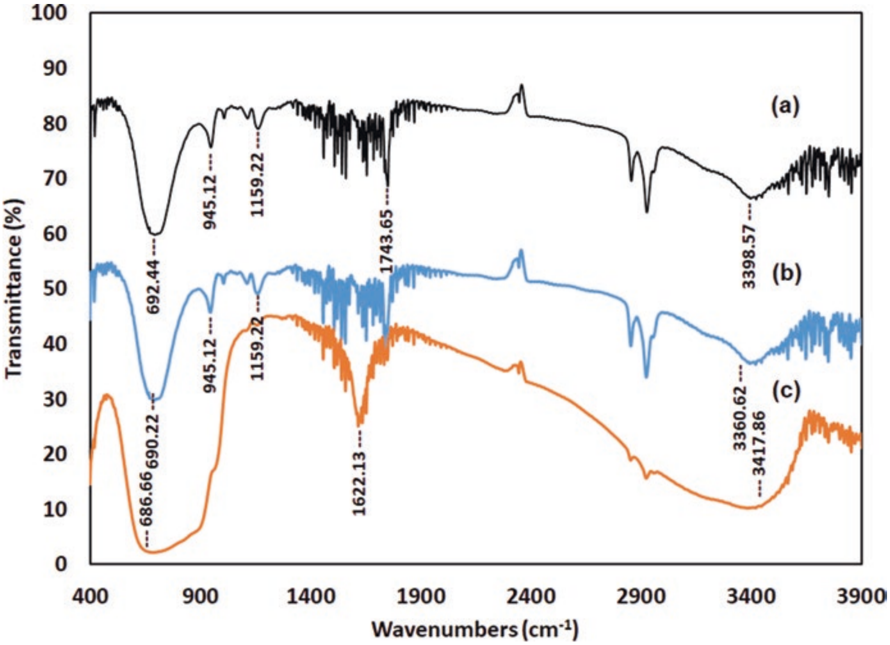


Fig. 3 FTIR spectrum of *A. galanga* aqueous extract (**b**), WO_3 (**a**) and IAA-WO_3 (**c**) nanoparticles

attributed to O–H stretching of alcohol and phenol groups. The peak at 945 cm^{-1} could be responsible for carboxylic group of O–H bend, respectively. The band 1159 cm^{-1} can be attributed to C–O stretch of ester and alcohol. These functional groups are responsible for the capping and reduction agent for the formation of WO_3

NPs. In the FTIR spectrum analysis of IAA-coated WO_3 NPs revealed that primary amine (1622 cm^{-1}) and phenol and alcohol (3417 cm^{-1}) function groups are capped in *Alpinia galanga*-mediated WO_3 NPs.

3.2 EDX Spectrum of WO_3 and IAA-WO_3 NPs

The element composition of WO_3 and IAA-WO_3 NPs is analyzed using EDX to confirm the purity of nanoparticles. WO_3 NPs have 73.20% of tungsten and 26.80% of oxygen, whereas IAA-WO_3 NPs have 69.38% of tungsten and 30.62% of oxygen (Fig. 4). The energy dispersion X-ray analysis refers to the strong signal of W and O that confirms the presence of tungsten oxide nanoparticles.

3.3 XRD Analysis of WO_3 and IAA-WO_3 NPs

Figure 5 shows XRD pattern of *Alpinia galanga*-mediated WO_3 NPs matched with monoclinic WO_3 (JCPDS No. 43-1035), confirming the presence of nanoparticle in crystalline nature. The strongest peak at 2θ value matches to the crystal plane of (002), (020), (200), (120), (022), and (202) of WO_3 NPs. The crystallographic angle of nanoparticles is responsible for (020) and (200) of monoclinic WO_3 NPs. This result revealed that the aqueous extract of plant extract is responsible for the morphology and monoclinic phase of WO_3 NPs. For IAA-WO_3 NPs, 2θ value of (101), (210), (104), and (211) corresponds to the crystal nature of monoclinic $W_{18}O_{49}$ (JCPDS No. 05-0392). Lie et al. (2012) reported the synthesis of monoclinic WO_3 and $W_{18}O_{49}$ NPs in the presence of ethanol and water, respectively. Ahmadi et al. (2014) explained that low concentration of precursor would regulate crystalline phase and surface morphology of WO_3 NPs. This investigation described the IAA-WO_3 NPs' crystalline nature with monoclinic phase ($W_{18}O_{49}$).

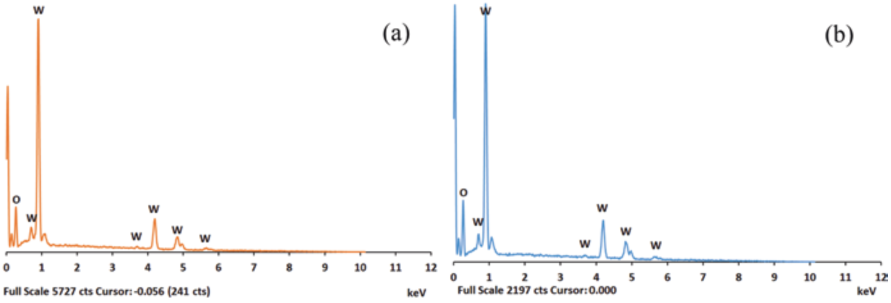


Fig. 4 EDX spectrum of green synthesized WO_3 (**a**) and IAA-WO_3 (**b**) nanoparticles

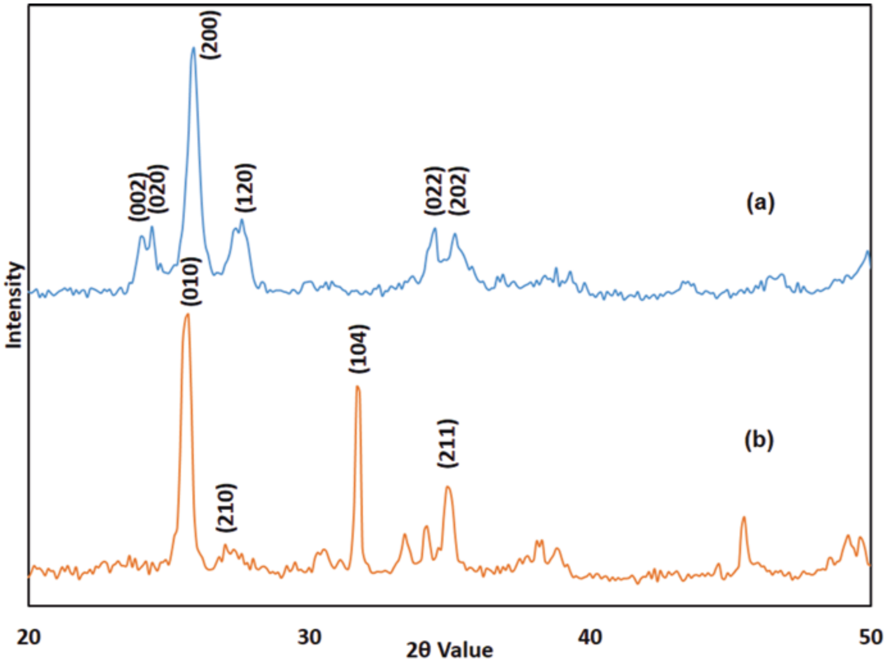


Fig. 5 XRD patterns of (**a**) WO_3 and (**b**) IAA-WO_3 NPs

3.4 *SEM and DLS Analysis*

Scanning electron microscopy (SEM) images of the WO_3 and IAA-WO_3 NPs showed surface morphology at various magnifications (Figs. 6 and 7). This figure suggested that WO_3 nanoparticles have a regular spherical shape, but IAA-WO_3 NPs are aggregated with irregular spherical shape. Dynamic light scattering analysis is used to determine average size distribution of WO_3 and IAA-WO_3 NPs (Fig. 8). The average size distribution of WO_3 was found to be 180–600 nm and that of IAA-WO_3 NPs was 85–180 nm. The DLS analysis confirmed that the average size of WO_3 and IAA-WO_3 NPs is 316 nm and 123 nm, respectively (Table 3).

3.5 *Effect of WO_3 and IAA-WO_3 NP Treatment on* Linum usitatissimum *Germination*

All treatments lead to the seed germination, showing that WO_3- and IAA-coated WO_3 NPs did not show any adverse effect on *Linum usitatissimum* seed germination. However, with increasing soaking time (day) there was a slight decrease in root lengths. Significantly, positive influence on root elongation and higher percentage

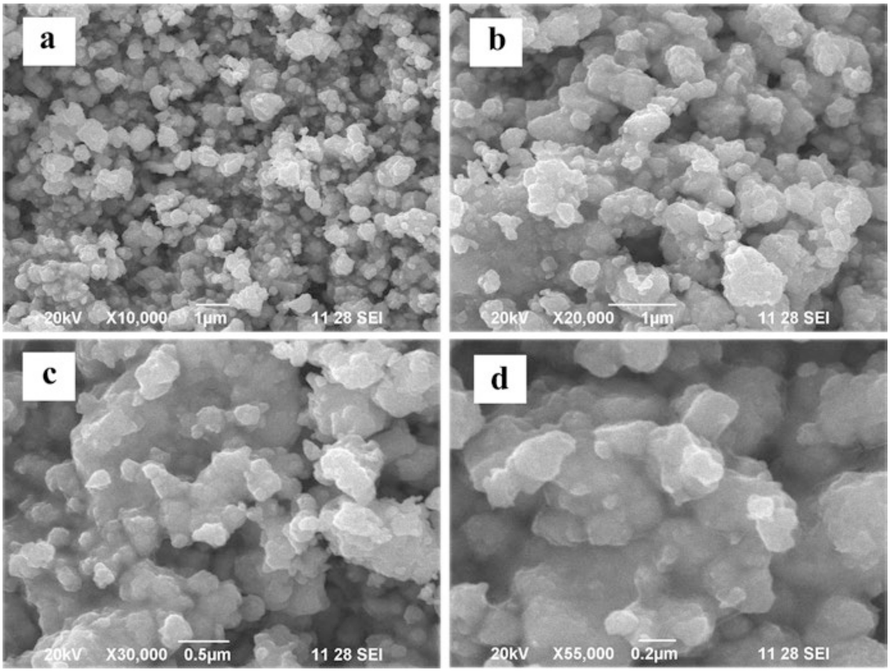


Fig. 6 Scanning electron microscope image of synthesized WO_3 nanoparticles with different magnifications

Table 3 The average size distribution of WO_3 and IAA-WO_3 NPs estimated by using dynamic light scattering

Nanoparticles	Size distribution (d nm)	Z-average size (d nm)	Width (d nm)	PdI
WO_3	180 to 600	316	76.13	0.003
IAA-WO_3	85 to 180	123	20.23	1.000

of seed germination were observed in IAA-WO_3 NPs on the first day of soaking (Table 4). IAA-WO_3 NPs on root elongation of *Linum usitatissimum* increase in lower concentrations (0.1 g/L) but decrease in higher concentrations (0.5, 1, and 2 g/L) when compared to WO_3 NPs.

IAA-WO_3 NPs were found to have less toxic effect on root elongation at the concentration of 0.1 g/L than green synthesized WO_3 NPs (Fig. 9b). However, 1 g/L concentration of IAA-WO_3 NP-treated seed shows better root length when compared to WO_3 NPs. Seed grown in the presence of WO_3 NPs showed 34% of germination whereas IAA-WO_3 NPs showed 54%. The relative toxicities are based on the germination index for the tested NPs (WO_3 > IAA-WO_3) (Fig. 9).

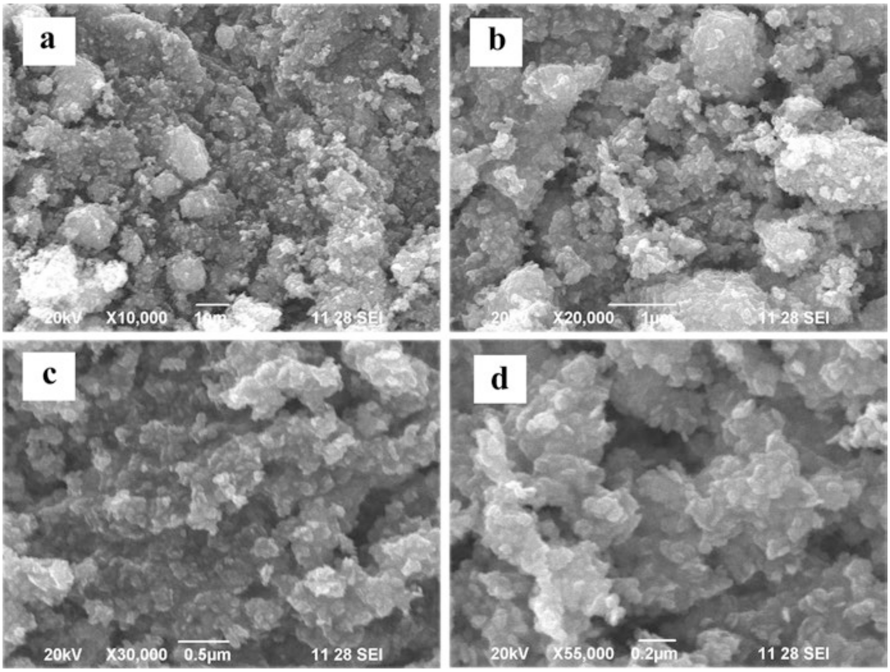


Fig. 7 Scanning electron microscope image of synthesized of IAA-WO_3 nanoparticles with different magnifications

Table 4 Effect of WO_3 and IAA-WO_3 NPs on germination by soaking method

Concentration	Day 1		Day 2		Day 3	
	WO_3	IAA-WO_3	WO_3	IAA-WO_3	WO_3	IAA-WO_3
0.1 mg/L	0.96 ± 0.25	3.86 ± 0.96	0.43 ± 0.15	1.53 ± 0.68	0.6 ± 0.20	0.53 ± 0.20
0.25 mg/L	1.1 ± 0.3	2.73 ± 0.47	0.96 ± 0.15	0.16 ± 0.15	0.1 ± 0.10	0.56 ± 0.15
0.5 mg/L	0.66 ± 0.20	3.23 ± 0.87	1.23 ± 0.68	1.3 ± 0.26	0.6 ± 0.10	1.3 ± 0.20
1 mg/L	1.26 ± 0.50	0.86 ± 0.20	1.13 ± 0.51	1.43 ± 0.90	0.2 ± 0.10	0.6 ± 0.10
2 mg/L	0.63 ± 0.25	2.63 ± 0.92	1.03 ± 0.35	0.96 ± 0.15	0.5 ± 0.10	1.4 ± 0.43
Control	2.8 ± 0.52		2.3 ± 0.26		0.3 ± 0.20	

Results are expressed on mean ± SD, $n = 3$

3.6 Effect of WO_3 and IAA-WO_3 NPs on Biochemical Parameter of Linum usitatissimum

Plant biometric parameters such as carbohydrate, protein, and reducing sugar are estimated in *Linum usitatissimum* treated at various concentrations of nanoparticles (Table 5). Higher level of carbohydrate content was obtained in IAA-WO_3 NPs (0.92 ± 0.40 mg/g), which was compared to WO_3 NPs (0.70 ± 0.18 mg/g) and control (0.63 ± 0.10 mg/g) at a concentration of 0.1 g/L. The protein content of

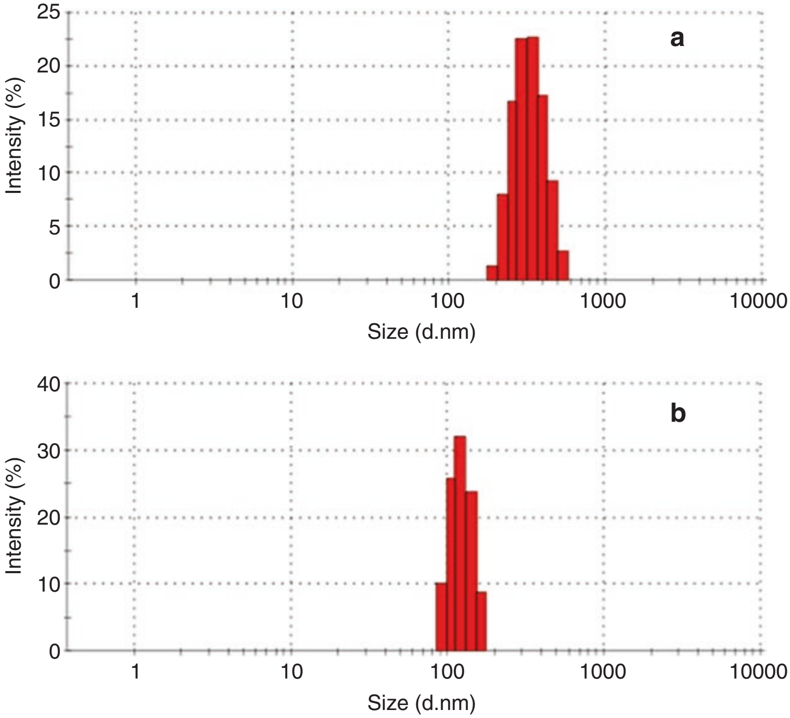


Fig. 8 DLS spectrum of (**a**) WO_3 and (**b**) IAA-WO_3 nanoparticles

Table 5 Comparison of WO_3 and IAA-WO_3 NPs on the biochemical parameter of flaxseed

Concentration	Carbohydrate (mg/g)		Protein (mg/g)		Reducing sugar (mg/g)	
	WO_3	IAA-WO_3	WO_3	IAA-WO_3	WO_3	IAA-WO_3
0.1 mg/L	0.70 ± 0.18	0.92 ± 0.40	0.71 ± 0.10	0.77 ± 0.12	0.54 ± 0.15	0.78 ± 0.09
0.25 mg/L	0.87 ± 0.23	0.58 ± 0.24	0.63 ± 0.09	0.73 ± 0.10	0.95 ± 0.54	1.14 ± 0.08
0.5 mg/L	0.69 ± 0.26	0.67 ± 0.31	0.58 ± 0.02	0.66 ± 0.05	0.70 ± 0.19	1.48 ± 0.36
1 mg/L	0.65 ± 0.32	0.87 ± 0.22	0.51 ± 0.08	0.60 ± 0.06	0.49 ± 0.19	1.31 ± 0.06
2 mg/L	0.73 ± 0.29	0.69 ± 0.17	0.49 ± 0.10	0.53 ± 0.05	0.69 ± 0.33	1.29 ± 0.22
Control	0.59 ± 0.12	0.63 ± 0.10	0.43 ± 0.03	0.45 ± 0.02	0.59 ± 0.07	0.59 ± 0.06

Data represented as mean ± SD, $n = 3$, and paired "*t*" test between WO_3 and IAA-WO_3 NPs

IAA-WO_3 and WO_3 NPs is more or less similar. The amount of protein present in WO_3 and IAA-WO_3 NP-treated plant showed higher level (0.71 ± 0.10 and 0.77 ± 0.12 mg/g) at a concentration of 0.1 g/L. Decreased protein level in seedling plant due to NP treatment leads to protein degradation. Similar trend of drop in reducing sugar was noticed in *Linum usitatissimum* with increased concentration of nanoparticles. IAA-WO_3 NPs showed maximum reducing sugar level of 1.48 ± 0.36 mg/g at a concentration of 0.5 g/L. Likewise, WO_3 NPs exhibited

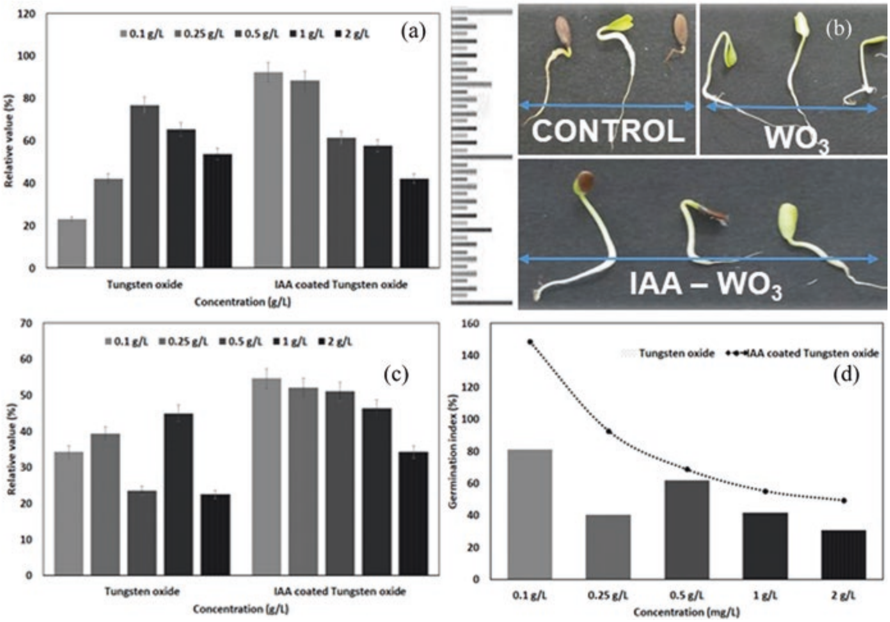


Fig. 9 Effect of WO_3 NPs and IAA-WO_3 NPs on (**a**, **b**) root elongation, (**b**) germination rate, and (**c**) germination index of flaxseed. Results are statistically significant ($P < 0.05$) and data represented as mean ± SD, $n = 3$

0.95 ± 0.54 mg/g of reducing sugar noticed at 0.25 g/L concentration. The reduction in carbohydrate, protein, and reducing sugar content of the experimented plants at higher doses (1 and 2 g/L) may be attributed to toxic level of nanoparticles causing consequent decline in growth (Agnihotri et al. 2014; Wasmi et al. 2014; Garavand et al. 2012).

3.7 Antifungal Activity

The antifungal assay of WO_3 and IAA-WO_3 NPs against *Aspergillus niger* (MTCC: 10180), *Fusarium oxysporum* (MTCC: 3327), and *Penicillium funiculosum* (MTCC: 4888) was determined by well-diffusion method. IAA- and *Alpinia galanga*-mediated WO_3 NPs showed an efficient antifungal activity against phytopathogens (Tables 6 and 7). Highest zone of inhibition was obtained in IAA-WO_3 NPs of *F. oxysporum* (12.46 ± 0.55 mm) at a concentration of 100 mg/mL and lowest zone of inhibition was found in *A. flavus* with a zone diameter of 7.43 ± 0.60 mm.

Likewise, WO_3 NPs show maximum zone of inhibition observed in *F. oxysporum* (9.63 ± 0.32 mm) and minimum zone of inhibition in *P. funiculosum* (8.40 ± 0.45 mm) at a concentration of 100 mg/mL. When compared with control and IAA-WO_3 NPs, antifungal activity of WO_3 NPs (Table 8) is not promising to prevent seeds from

Table 6 Antifungal activity of IAA-WO_3 nanoparticles against plant pathogen

Pathogens	Different concentrations of WO_3 nanoparticles 50 mg/L	100 mg/L	Control (amphotericin B 10 mg/ml)
A. flavus	5.5 ± 0.50	7.43 ± 0.60	7.23 ± 0.25
F. oxysporum	8.20 ± 0.26	12.46 ± 0.55	10.98 ± 0.21
P. funiculosum	6.17 ± 0.17	9.84 ± 0.26	9.30 ± 0.26

The resulted zone of inhibition (mm) is expressed in mean ± SD

Table 7 Antifungal activity of WO_3 nanoparticles against plant pathogen

Pathogens	Different concentrations of IAA-WO_3 nanoparticles 50 mg/L	100 mg/L	Control (amphotericin B 10 mg/ml)
A. flavus	6.76 ± 0.25	8.90 ± 0.10	7.44 ± 0.50
F. oxysporum	6.50 ± 0.50	9.63 ± 0.32	10.54 ± 0.25
P. funiculosum	5.23 ± 0.30	8.40 ± 0.45	9.10 ± 0.21

The resulted zone of inhibition (mm) is expressed in mean ± SD

Table 8 ANOVA analysis of variance for the data on inhibition zone of WO_3 and IAA-WO_3 nanoparticles against plant pathogen

Pathogens		Sum of square	Mean square	F	Sig.
A. flavus	Between group	18.053	4.513	30.089	0.000∗
	Within group	1.50	0.150		
	Total	19.553			
F. oxysporum	Between group	65.051	16.263	105.895	0.000∗
	Within group	1.536	0.154		
	Total	66.587			
P. funiculosum	Between group	48.047	12.012	126.858	0.000∗
	Within group	0.947	0.095		
	Total	48.994			
Control	Between group	25.938	8.646	115.205	0.000∗
	Within group	0.600	0.075		
	Total	26.539			

All the results are statistically significant (P-value ≤ 0.05) using Tukey test
Sig: Significant, $*P < 0.05$

diseases. Kumar et al. (2015) described the synthesis of WO_3 NPs with monoclinic structure by simple precipitation method and declared that NPs have efficient antimicrobial property. Kulal et al. (2016) explained that WO_3 NPs had lower inhibition ability against pathogenic microorganisms. However, WO_3-doped or -coated nanocomposite will enhance antimicrobial activity.

4 Conclusion

This study reported WO_3 NPs synthesized by precipitation method using *Alpinia galanga* leaf extract acting as a capping and reducing agent with sodium tungstate precursor. Synthesized *Alpinia galanga*-mediated WO_3 NPs combined with IAA enhanced the growth of *Linum usitatissimum* plant. The synthesized WO_3 and IAA-WO_3 NPs were monoclinic phase (WO_3 and $W_{18}O_{49}$) with z-average size of 316 and 123 d nm, respectively. The different concentrations of WO_3 and IAA-WO_3 NP effect on germination and root elongation of flaxseed were studied. We observed that flaxseed germination at the lowest concentration (0.1 and 0.25 g/L) of IAA-WO_3 nano-suspension solution assured good root growth and biochemical parameters compared to WO_3 NPs. In addition, it shows promising antifungal activity against *F. oxysporum, A. flavus*, and *P. funiculosum*. Thus, we conclude that *Alpinia galanga*-mediated tungsten oxide nanoparticles coated with indole-3-acetic acid can be used as a nanofertilizer for the growth of vegetative crop and control of fungal related diseases.

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Phytoremediation of Contaminated Soils Using Trees

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Contents

1 Introduction

Heavy metal pollution problem has increased at a great pace recently. Development in industrial activities, expanded utilization of pesticides and herbicides, and fossil fuel consumption often prompt soil pollution with potential serious health risks to the human population (Asgari Lajayer et al. 2017a, b). It has affected the quality of soil to an extreme level as heavy metals are nonbiodegradable and they tend to accumulate in the environment causing a major threat to the living organisms (Asgari Lajayer et al. 2019). A recovery technique for soils polluted with metals that has

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gained vast popularity is phytoremediation since it is monetarily proficient and safe for the environment (Pandey et al. 2019).

Phytoremediation has numerous advantageous highlights that make it a proper and successful technology (Asgari Lajayer et al. 2019). In recent years, the utilization of trees for phytoremediation has grabbed a lot of attention because of their capacity to thrive on soils which are nutrient deficient, as they display high biomass efficiency, profound root frameworks, financially viable secondary usage, and specific aggregation of contaminants (Liu et al. 2013). In addition to this, tropical tree species display fast development and are generally perennial. However, not much research has been done on the utilization of local tree species from tropical and subtropical regions for the phytoremediation of soils polluted with metals (Pereira et al. 2010). Various traits like high biomass and economic importance make trees suitable for phytoremediation but in order to become compelling for the utilization in phytoremediation, they should accumulate and tolerate high heavy metal concentrations (Pulford et al. 2001). They are high biomass makers and for specific species, for example, *Salix*, the absence of reported toxic effects in trees demonstrates that their resistance mechanisms may enable them to withstand greater metal concentrations than agricultural crops. The wide genome of trees and facultative resistance, for example, the redistribution of roots to less defiled zones of soil, permits their survival not chosen for metal resilience on contaminated soils. Bioavailability of metals to trees and consequent metal amassing in tree tissues can change enormously as per the wellspring of metal tainting and site conditions (Pulford and Watson 2003). Thus, there is lot of potential in trees for the phytoremediation of heavy metal-contaminated sites. Mobility of heavy metals within a tree displays different behavioral patterns; for example, Pb and Cr are generally immobilized in roots, whereas Ni and Cd get translocated to the aerial parts easily (Pulford and Watson 2003). There are several advantages of using trees for the phytoremediation purpose like phytostabilization of toxic metals in roots, and soil loss is prevented which is caused by erosion as large biomass of roots of trees bind the soil. Trees also lower the leaching losses in soil. Hence, heavy metal-contaminated sites can be physically stabilized by growing trees.

2 Phytoremediation: A Green Approach for Reducing the Pollution Load of Environment

With fast economic development, urbanization, and industrialization, environmental pollution caused by various toxins at an alarming rate, thereby overwhelming the self-purification limit of natural media, is enhancing global anxiety (Luo et al. 2018). Anthropogenic heavy metals (HMs) mainly originate from traffic, waste disposal, sewer sludge, release of dust, aerosols, fly ash from the metal industries, and utilization of phosphate fertilizer which usually contains cadmium as a contamination (Sun et al. 2016; Zhang et al. 2014). Likewise, the mobilization of HMs by man for extraction from ores and handling for assorted utilizations has prompted the spread of these elements into the earth. In the environment, concentrations of HMs are increasing manifold on a yearly basis (Govindasamy et al. 2011). Since HMs do not get dissolved, they aggregate in the environment and pollute the food chain (Ali et al. 2013). With respect to the above, it can be deduced that people get affected not only through contaminated air but also through other parts of polluted environment. The concentration of HMs in the environment must be controlled as it acts as an influencing health factor (Jiang et al. 2017; Sarwar et al. 2017). Table 1 gives bad effects of special HMs on human health.

According to Table 1, it can be seen that HMs pose toxic effects on human well-being and contaminate the food chain; hence special consideration is needed in this regard. Numerous HMs and metalloids are toxicant and can cause harmful impact and extreme troubles even when they are present at very meager concentrations (Arora et al. 2008; Memon and Schröder 2009; Ali et al. 2013). Today, researchers' welcome to indirect methods of monitoring, such as the reaction of living beings to

Table 1 Negative impacts of HMs on human health

HMs	Negative impacts	References
Pb	Several problems like loss of short-term memory, hindered development, diminished insight, coordination issues and disability in learning, renal failure; increased hazard for development of cardiovascular disease is caused in children due to Pb poisoning	Salem et al. (2000); Padmavathiamma and Li (2007); Wuana and Okieimen (2011); Iqbal (2012)
Cu	Brain and kidney damage, chronic anemia, irritation in intestine and stomach, and liver cirrhosis have been found to be caused due to enhanced levels of Cu in the body of living organisms	Salem et al. (2000); Wuana and Okieimen (2011)
Cr	Causes hair loss	Salem et al. (2000)
Ni	Lung, nose, throat, and stomach cancer is caused by its inhalation. It is known to be immunotoxic, genotoxic, hematotoxic, reproductive toxic, neutrotoxic, pulmonary toxic, hepatotoxic, and nephrotoxic and also known to cause allergic dermatitis also called as nickel itch	Salem et al. (2000); Khan et al. (2007); Das et al. (2008); Duda-Chodak and Blaszczyk (2008); Mishra et al. (2010)
Zn	Fatigue and dizziness are caused due to its overusage	Hess and Schmid (2002)
Cd	Cd caused chronic anemia and renal failure. It is known to be mutagenic, carcinogenic, endocrine disruptor, and teratogenic and inhibits regulation of calcium in biological systems	Degraeve (1981); Salem et al. (2000); Awofolu (2005)
As	Being a phosphate analogue, it interferes (as arsenate) with fundamental cellular processes like ATP synthesis and oxidative phosphorylation	Tripathi et al. (2007)
Hg	Damage to lungs, brain, and kidney, restlessness, sleep deprivation, autoimmune diseases, depression, drowsiness, fatigue, baldness, irritability, loss of memory, recurrent infections, anxiety, vision disturbances, tremors, ulcers, and temper outbursts	Neustadt and Pieczenik (2007); Ainza et al. (2010); Gulati et al. (2010)

pollutants, is rising due to time consumption, high costs, and invalid information (Abril et al. 2014; Boquete et al. 2014). Cleanup approaches have been improved for the removal of HMs, but most of these methods are expensive and sometimes not environmental friendly effects. Some cleanup approaches such as soil washing techniques for removing metals from soil have deleterious effects on the soil physical and biological properties (Pulford and Watson 2003). There are some methods for limiting the solubility of metals, that is, immobilization methods. Phytoremediation is a low-cost technique in which plants are utilized for reducing the concentration of HMs from the contaminated soils; thus, it is a sustainable method for the soil quality. Phytoremediation methods have elaborated in numerous literatures or articles (Tangahu et al. 2011). This term is a combination of two words: Grecian prefix phyto (in the sense of the plant) and Latin root remedium (meaning to eradicate an evil) (Tangahu et al. 2011; Ali et al. 2013). Several researchers have defined phytoremediation in their own way and some of them are listed in Table 2.

In general, as defined by the abovementioned researchers, phytoremediation is an emerging technology which enhances the quality of environment by reducing the pollutants from the contaminated environment by selected plants. The research and development studies for phytoremediation have been conducted mostly during the last two decades (1990 onwards), and thus it is a new technology relatively. The concept of phytoremediation was explained as phytoextraction by Chaney (1983). The different phytoremediation strategies for decontamination of polluted environment are presented in Table 3.

Though the traditional methods for soil remediation such as extraction, chemical leaching, vitrification, solidification, filtration, and thermal treatment are less time consuming they are much expensive and they negatively affect the soil properties and attributes. On the other way round, phytoremediation is a low-cost alternative for the same with minimum impact on soil properties. This definition for the phytoremediation originated from the plants that can take up the high content of HMs in the aboveground tissues, which are called hyperaccumulator plants (Baker et al. 1994; Chaney et al. 1997). Hence, plant tissues can sequester the HMs in the environment (Alahabadi et al. 2017); additionally, HMs and other airborne pollutants can be adsorbed by other tree parts (Sawidis et al. 2011; Miri et al. 2016).

Phytoremediation is a new, more affordable, compelling, environment- and eco-friendly remediation strategy. Table 4 lists the major advantages of phytoremediation.

Economically, threefold benefit can be obtained by utilizing the technique of phytoremediation for polluted lands (Vangronsveld et al. 2009) as shown in Fig. 1.

3 Phytoremediation of Contaminated Soils Using Trees

Today, globally, the study is centered on phytoremediation by trees that are welcomed. Aghaalikhani et al. (2017) studied the utilization of poplar (P) and hazelnut shell (HS) phytoremediation pruning (PHYP). Poplar showed the capability to absorb pollutants (HMs) from the soil in which they are cultivated. The samples

Table 2 List of some definitions of phytoremediation

No.	Definition of phytoremediation	References
1	The utilization of plants, including trees and grasses, to evacuate, demolish, or sequester perilous contaminants from media, for example, air, water, and soil	Vara Prasad and Oliveira Freitas (2003)
2	The utilization of vascular plants to expel toxins from the environment or to render them innocuous. The engineered utilization of green plants to expel, contain, or render innocuous such contaminants as toxic heavy and trace metals, radioactive and organic compounds in soil or water	Bhattacharya et al. (2006)
3	The utilization of plants to enhance debased environments	Moreno et al. (2008)
4	The utilization of plants to remediate harmful synthetic compounds found in tainted soil, muck, dregs, groundwater, surface water, and wastewater. The utilization of plants to remediate harmful synthetic compounds found in tainted soil, sludge, sediment, waste, ground, and surface water	Rodriguez et al. (2005)
5	An emerging technology utilizing specially screened and engineered metal-accumulating plants for environmental cleanup	Liu et al. (2000)
6	Phytoremediation in general implies the utilization of plants (with their associated microorganisms) to expel, debase, or balance out contaminants	Van Ginneken et al. (2007)
7	Phytoremediation is the term assigned for a set of technologies that utilize different plants as a regulation, destruction, or an extraction strategy. Phytoremediation is an emerging method that utilizes different plants to degrade, extract, contain, or immobilize contaminants from soil and water	US (2000)
8	This definition incorporates all plant-impacted biological, chemical, and physical processes that help in the uptake, accumulation, debasement, and metabolism of contaminants, either by plants or by free-living organisms that comprise the plant rhizosphere	Negri et al. (1996)

PHYP, HS, and P have likewise been examined so as to get the possible content of alkali and HMs. PHYP, since utilized in phytoremediation, is relied upon to contain a higher amount of HMs. Results demonstrated that HMs are found in exceptionally low amounts in all the three samples; exclusion is indicated by Sr and Zn which are present in higher amounts in the sample PHYP. In PHYP, Sr is ten times and Zn is around two times greater as compared to P. Alahabadi et al. (2017) conducted a study in which screening and comparison were done among 14 tree species in terms of their potential to sequester four airborne and soilborne HMs. In all the 14 tree species, the ability to accumulate HMs in bark and leaf with the same HM condensation was studied. Based on Comprehensive Bioconcentration Index (CBCI) *Wisteria sinensis* and *Pinus eldarica* had the maximum capacity to accumulate HMs from soil, and values of metal accumulation index (MAI) demonstrated that *Nigral morus* and *Morus alba* had the greatest capacity to amass HMs from ambient air. In *Pinus eldarica* leaves, higher bioconcentration factor (BCF) values for Pb, Zn, Cd, and Cu were found than in *Morus alba*, and in barks the higher values of BCF for Pb, Zn, Cd, and Cu were found in *Buxus hyrcana*, *Morus alba*, and *Nigral morus*

Table 3 Brief explanation for various methods included in phytoremediation technology

Method	Brief explanation	References
Phytoextraction	Pollutants are accumulated in harvestable biomass, i.e., shoots	Rafati et al. (2011); Tangahu et al. (2011)
Phytofiltration	Plants sequester contaminants from polluted water	Mesjasz-Przybyłowicz et al. (2004); Sangeeta and Maiti (2010)
Phytostabilization	The bioavailability and mobility of contaminants in soil are limited by plant roots	Wuana and Okieimen (2011); Yoon et al. (2006)
Phytovolatilization	Pollutants are interchanged to their volatile forms and subsequently released into the atmosphere	Padmavathiamma and Li (2007)
Phytodegradation	Plant enzymes degrade the organic xenobiotics within plant tissues	Vishnoi and Srivastava (2007)
Rhizodegradation	Rhizospheric microorganisms degrade the organic xenobiotics in the rhizosphere through this technique	Kuiper et al. (2004); Yadav et al. (2010)
Phytodesalination	In this method, halophytes remove excess salts from saline soils	Manousaki and Kalogerakis (2011); Sakai et al. (2012)

Table 4 Advantages of phytoremediation

No.	Advantages of phytoremediation	References
1	Various combinations of organic and mineral treatments are amenable in phytoremediation method	Moosavi and Seghatoleslami (2013)
2	This green technology is appropriate for vast zones in which different methodologies would be costly and ineffective	Vara Prasad and Oliveira Freitas (2003); Vidali (2001)
3	This technology can be utilized either as an ex situ or an in situ practice	Henry (2000); Laghlimi et al. (2015)
4	It creates “green belts” by utilizing tress, and thus proves to be aesthetically beneficial as tress are socially and psychologically valuable for everyone	Ghosh and Singh (2005); Lewis (2006)
5	It can possibly treat sites contaminated with more than one type of toxicant. Besides, the expansion of pollutants to air and water is diminished by inhibiting leaching and soil disintegration that may result from wind and water action	Hegedusova et al. (2009); Pivetz (2001)
6	It is a low-cost process compared to traditional cleanup methods. For instance, the expense of cleaning up one acre of sandy loam soil with a tainted depth of 50 cm with plants was evaluated at \$60,000–\$100,000 when compared to \$400,000 for the traditional excavation and disposal techniques	Laghlimi et al. (2015); Raskin and Ensley (2000)
7	In this technique, risk of contamination spread is reduced as disposal sites are not required and thus transport of polluted media is not needed	Moosavi and Seghatoleslami (2013); Tangahu et al. (2011)
8	Highly specialized staff not required; relatively easy to perform	Laghlimi et al. (2015)

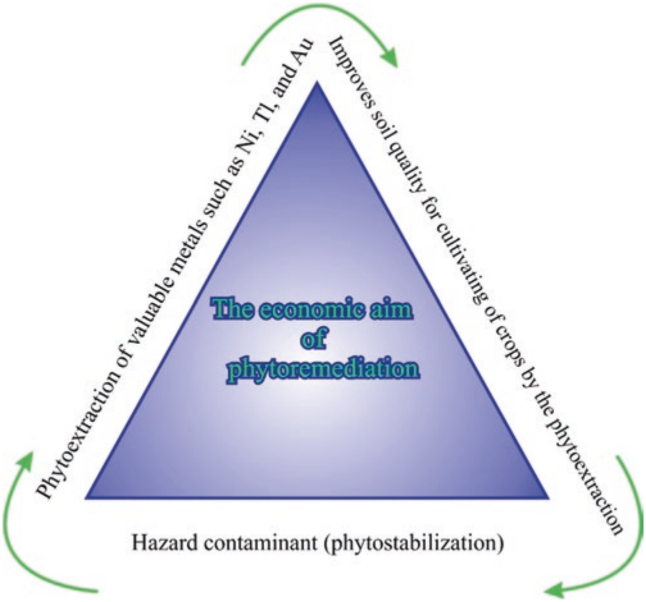

Fig. 1 Threefold benefits by phytoremediation of polluted lands

species. Hence, these species could be used as good bioaccumulators for the HMs mentioned above. Their high aggregation potential can likewise represent their utilization ability for the biomonitoring of the pollution of HMs in the living environment. Zhao et al. (2014) developed CBCI and it was applied to determine the ability of trees for comprehensive uptake of various HMs. In Fig. 2 CBCI is presented for 14 tree species in leaves and bark (Alahabadi et al. 2017). CBCI calculated with Eqs. (1) and (2):

$$\mu(x) = \frac{x - x_{\min}}{x_{\max} - x_{\min}} \tag{1}$$

where x is the bioconcentration factor (BCF) of a specific metal, and $x_{\min}$ and $x_{\max}$ are the minimum and maximum values of the BCF for the specified metal, respectively. The maximum and minimum values can be one and zero, respectively, which represent the most elevated and most reduced comprehensive amassing variables of different HMs. Eventually, CBCI was evaluated with Eq. (2):

$$\text{CBCI} = \frac{1}{N}\sum_{i=1}^{N} \mu i \tag{2}$$

where N represents the total number of HMs analyzed, and mi is $m(x)$ of metal i. In Fig. 3 MAI is presented for 14 tree species in leaves and bark (Alahabadi et al. 2017).

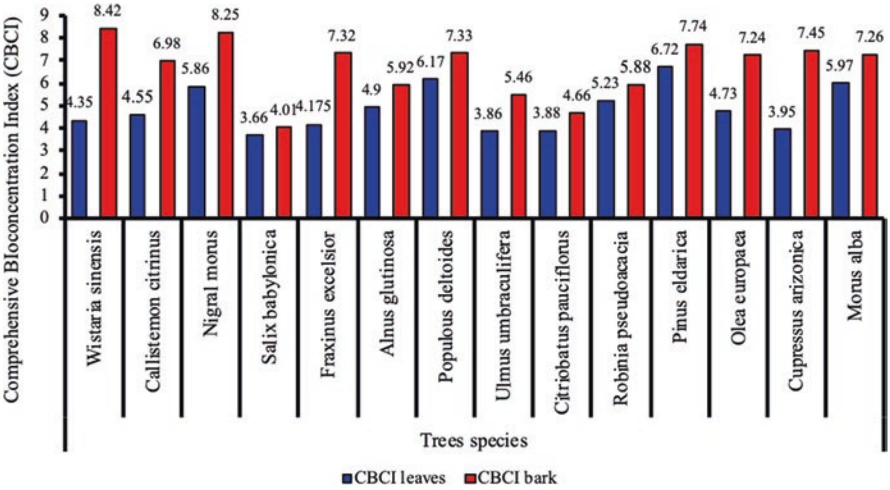

Fig. 2 CBCI for 14 tree species in leaves and bark

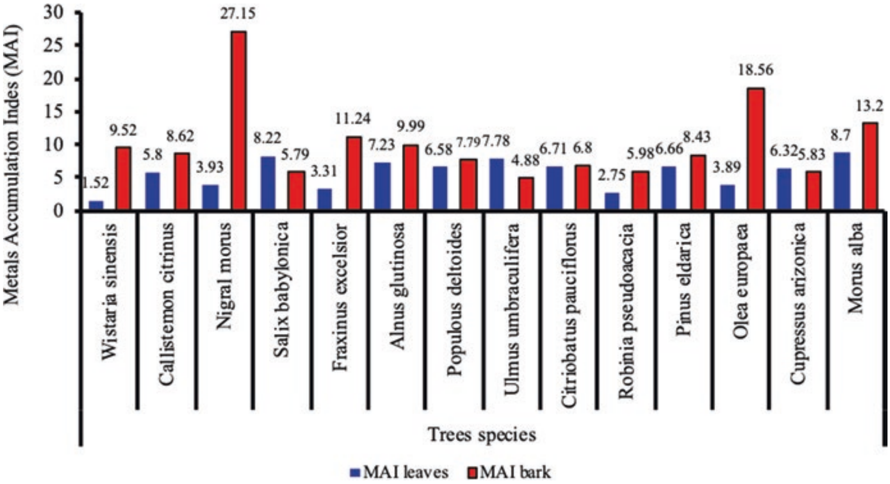

Fig. 3 MAI for 14 tree species in leaves and bark

Liu et al. (2007) presented Metal Accumulation Index (MAI). The potential of various tree species to accumulate HMs differs. Hence, the ability of different tree species for HM accumulation was assessed and compared by applying MAI.

MAI was computed with Eqs. (3) and (4):

$$\mathrm{MAI} = \frac{1}{N}\sum_{j=1}^{N} Ij \tag{3}$$

$$Ij = \frac{x}{\Delta x} \tag{4}$$

In the relationships provided, N represents the total number of HMs analyzed, and Ij is the sub-index for the variable j. Ij was evaluated by dividing the mean concentration value (x) of every HM by their standard deviation (Δx).

Figure 4 presents the top ten countries universally ranked by the number of emissions in bioremediation area in the distance, 2005–2015, and also the ranking of Romania and Brazil and the ranking of Pakistan, Bangladesh, and South Africa, identified as countries with intense soil pollution and interested in bioremediation and phytoremediation. One can recognize from Fig. 2 the top countries that make the search in this area, China competing strongly with the United States for the leading search in this area (Amărioarei et al. 2017).

In global prospects, research on phytoremediation has grabbed a lot of attention and work on it is being conducted in almost every continent. However, majority of the study has been pursued in the United States, Asia, and Africa. Promising results as an innovative cleanup technology have been demonstrated by the knowledge of phytoremediation. But it is still in a developmental phase and more intensive investigation is required to enhance the level of understanding and science behind this technology (Sharma and Pandey 2014).

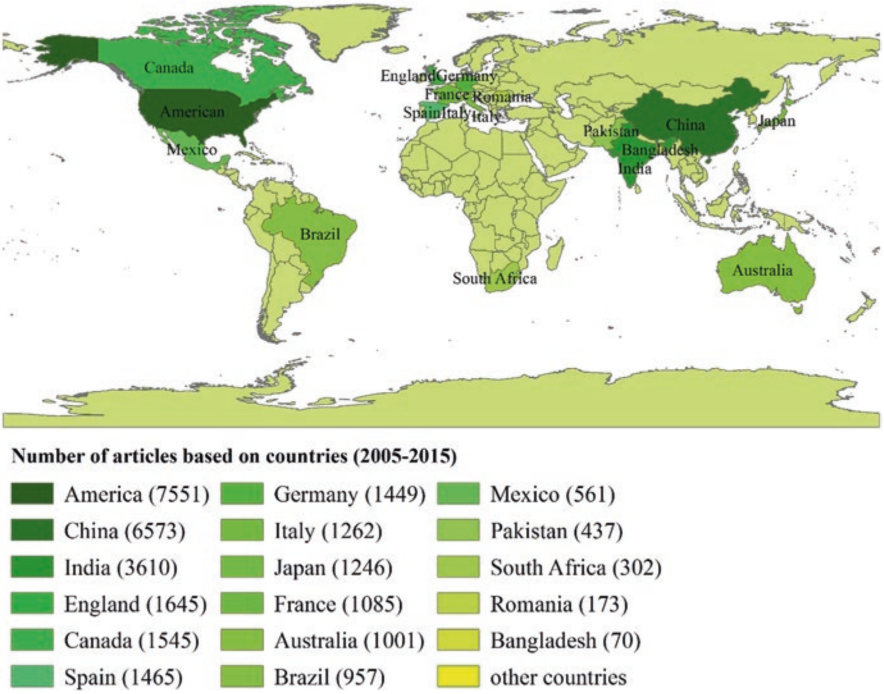

Fig. 4 World map with the number of publications for any country

Zhao et al. (2014) conducted a research to determine and compare the accumulation potential of Zn, Pb, Cd, and Cu in some wild woody plants (18 species) in Hunan area in China. They reported that large Pb sequestration potential is demonstrated by native species *P. fortunei* and *B. papyrifera* whereas the endemic species *Z. insignis* has large Cd and Zn uptake potential. Results revealed that *A. fruticosa* L. *indica*, *B. papyrifera, P. fortunei*, *Z. insignis* and *Q. fabri* have significantly higher metal accumulation potential. Studies of researchers have shown that some mangrove species are able to accumulate HMs in roots, stems, and leaves and in special species like *Avicennia marina*, HMs like Pb and Cu can be accumulated greater than in other species (Heriyanto and Subiandono 2011; John and Waznah 2011; MacFarlane and Burchett 2002). For example, Ika Harlyan et al. (2015) conducted a study to specify whether different absorption levels are present for unnecessary (Pb) and essential (Cu) HMs in *Avicennia marina* from various places, based on the distance from the source of contaminants (downstream) toward the mouth of Porong River Estuary at Sidoarjo. Results revealed that uptake of Pb in leaves and roots ranged from 0.004 to 0.019 ppm and from 0.0044 to 0.139 ppm, respectively, while it ranged from 0.0560 to 0.0660 ppm in the sediment. The results showed that the mangrove *Avicennia marina* has the potency to accumulate HMs Pb and Cu. The concentration of both HMs (Pb and Cu) in sediment is greater than in the mangrove (roots and leaves). According to the statistical test, no significant difference in the level of accumulation of heavy metals Pb and Cu in different locations from downstream to estuary on the mangrove *Avicennia marina* was recorded. Qados (2015) studied the level of tolerance to Cd and Pb pollution with phytoremediation by native tree species in the kingdom of Saudi Arabia. Results revealed that a significant reduction in vegetative growth parameters and photosynthetic pigments was recorded at the maximum level of contamination with Cd and Pb whereas proline in plant tissues increased with increasing level of Pb or Cd in the soil up to a certain level. Activity of catalase and peroxidase was recorded to enhance with increasing level of pollutants and then a decline in the activity of both was observed at the maximum level of Cd and Pb. According to results obtained, *A. saligna* was recorded to be the most tolerant species to Pb and Cd pollution and it was followed by *E. rostrata* whereas lowest level of tolerance to Pb and Cd was shown by *C. erectus*.

4 Improving Phytoremediation Efficiency of Trees Using Mobilization of Contaminant

Various mechanisms like chelation, solubilization, and desorption can be utilized to mobilize the pollutants as these reactions rearrange the contaminants from solid to liquid phase (Table 5); hence bioavailability of the same is enhanced (Bolan et al. 2014).

Soluble HM chelate complexes can be formed with the help of chelating agents, as they have greater affinity for HM ions; hence solubilization of HMs in soil can be enhanced. By acquiring knowledge about various agents that can positively affect the

Table 5 Summary of latest work done to assess the potential role of soil amendments in the mobilization of HMs in soils

Amendments	HMs	Results	References
Chelating/complexing agents			
EDTA (0.1 M, 0.001 M)	Cu	Enhanced mobilization and uptake of Cu in plants	Thayalakumaran et al. (2003)
EDTA, KI, citric acid	Hg	Increase in plant uptake for Hg was recorded. Maximum leaching was observed by EDTA	Smolińska and Król (2012)
EDTA	Pb	Pb uptake by plants increased due to enhancement in its mobility	Gabos et al. (2009)
EDTA, EDDS	Cu	Cu uptake was enhanced by both EDTA and EDDS. But higher Cu concentration in soil was recorded in EDTA treatment and it caused a significant reduction in the growth of plants	Ultra et al. (2005)
EDTA, EDDS	Cu	More efficient phytoextraction was observed by EDDS although increase in Cu uptake by plants was recorded in both	Zeremski-Škorić et al. (2010)
EDDS, MGDA	Pb, Zn	Although both Pb and Zn were mobilized still Pb uptake by plants was enhanced whereas Zn remained unaffected	Cao et al. (2007)
EDTA, citric acid	Cd, Cu, Pb, Zn	Enhancement in accumulation of HMs in shoot portion was recorded as there was an increase in their mobility in the soil	Sun et al. (2009)
EDTA, EDDS	Cu, Pb	Although Cu leaching was recorded higher than Pb yet no significant uptake was observed due to chelate's addition in soil	Karczewska et al. (2011)
EDDS (0.078 M)	Cu	Solubilization of HMs was limited up to 20 cm topsoil and after 1 day of application, soluble Cu was significantly increased in the top 5 cm soil layer Results concluded that in field conditions, potential leaching with EDDS can be controlled	Wang et al. (2012)
Oxalic acid, EDTA, NTA, EDDS, citric acid	As, Cr, Cu	Most significant results were recorded with EDDS, EDTA, and citric acid for Cu, Cr, and As, respectively	Almaroai et al. (2013)
EDTA (0.0025 M), citric acid	Cr, Ni, Ca, Fe, Mg, Al, Mn, Zn	Significant leaching (dissolution) of all metal (loids) was recorded by addition of chelants. EDTA forms more stable complexes with HMs which leads to more leaching of major elements and Ni than citric acid; Cr is significantly mobilized by addition of citric acid compared to EDTA; this may be attributed to a substitution reaction with Cr(VI)	Jean-Soro et al. (2012)
Citric acid	Cd, Pb	Phytoextraction of Cd and Pb along with antioxidative responses in *S. nigrum* was enhanced by addition of citric acid	Gao et al. (2012)

(continued)

Table 5 (continued)

Amendments	HMs	Results	References
Tartaric acid, citric acid	Cd, Pb, Zn	Citric acid addition enhanced mobilization of Cd, Pb, and Zn by 71%, 181%, and 112%, respectively, while tartaric acid increased mobilization 70%, 155%, and 135% of the same	Veselý et al. (2012)
Humic acid, EDTA	Cd	Plant uptake enhanced significantly	Evangelou et al. (2004)
CCA, EDTA	Zn, Pb	Pb uptake was enhanced in shoot significantly by addition of CCA but not of Zn	Li et al. (2005)
NTA, HEIDA (5–500 µM)	As, Cu, Zn	Uptake of As and Cu in vetiver and maize was enhanced 3–4 times by application of HEIDA in Cu-amended soil and NTA in As-amended soil at the rate of 20 mmol/kg whereas NTA treatment (20 mmol/kg) enhanced 37- and 1.5-fold of Zn uptake in shoots of vetiver and maize, respectively	Chiu et al. (2005)
EDTA, NTA, picolinic acid	Th(IV)	Significant formation and solubilization of Th to silica were demonstrated by application of EDTA, NTA, and, to a lesser extent, picolinic acid as they decreased the sorption of Th to silica	Reinoso-Maset et al. (2012)
EDDS, DTPA	Se	By application of 7.5 mmol/kg EDDS and 1.0 mmol/kg DTPA, Se removal was further enhanced 12- to 20-fold, respectively	Esringü and Turan (2012)
Organic amendments			
Poultry manure	Cd, Cu	In a 3-year study, available Cd concentration was increased by application of poultry manure, contrary to Cu	Hanč et al. (2008)
Rice straw, clover	Cd	Both amendments enhanced the Cd concentration in *Sedum plumbizincicola* but no significant increase was observed in soil	Wu et al. (2012)
Biochar, green waste compost	As, Cd, Cu, Zn	Addition of both amendments enhanced Cu and As concentrations in soil solution greater than 30-fold whereas a decline in Zn and Cd concentrations was recorded	Beesley et al. (2010)
Biochar	As	As concentration in soil pore water by addition of biochar but it diminished the uptake of the same by tomato plants	Beesley et al. (2013)
Biochar, sewage sludge	Cu, Ni Pb, Zn	Biochar treatment enhanced leaching of Cu, Ni, and Zn considerably greater than sewage sludge treatment. Plant uptake of Ni, Zn, Cd, and Pb was only slightly reduced by sewage sludge samples compared to biochar samples	Méndez et al. (2012)

(continued)

Table 5 (continued)

Amendments	HMs	Results	References
Sewage sludge	Cu	Degree of weathering affected the availability of Cu in soil solution and plants by treatment of sewage sludge. Enhanced solubility and uptake of Cu were observed when sewage sludge was applied in tailings where there was low level of sulfide oxidation	Forsberg et al. (2009)
Sewage sludge	Cd, Pb, Ni	By application of sewage sludge, concentration of Cd, Pb, and Ni in mung bean plants surpassed the permissible limits	Singh and Agrawal (2007)
Sewage sludge	Cd, Cu, Cr, Ni, Pb, Zn	Uptake of all the HMs was increased in *Beta vulgaris* plants by application of sewage sludge. Cd, Zn, and Ni concentrations were greater than the permissible limits	Singh and Agrawal (2007)
Sewage sludge, compost	Cd, Cu, Ni, Pb, Zn	Higher uptake of HMs in plant parts along with increase in dry matter was recorded by addition of both the amendments but did not exceed the permissible limit	Kandil et al. (2012)
Manure, sewage sludge, compost	Cd, Cu, Mn, Ni, Pb, Zn	Increase in HM concentration, but within permissible limits, was recorded in grains and seeds of spring triticale and spring rape by addition of amendments	Izewska (2009)

CCA coated chelating agent, *DTPA* diethylenetriamine penta acetate, *EDDS* ethylene diamine-*N*,*N*'-disuccinic acid, *EDTA* thylene diamine tetra-acetic acid, *HEIDA* hydroxyethylimino diacetic acid, *KI* potassium iodide, *MGDA* methyl glycine diacetic acid, *NTA* nitrilotriacetic acid

uptake mechanism of HMs, the efficiency of HM uptake by trees can be significantly enhanced. By addition of various biodegradable physicochemical agents like micronutrients and chelating agents and also by stimulating the HM uptake capability of the microbial community in the rhizospheric region of plants and trees, bioavailability of HMs can be enhanced leading to increase in their uptake by power crops (Tangahu et al. 2011). The outcomes are consistent with past investigations and give a valuable source to the enhancement of phytoremediation technology.

Also, bioavailability of metals can be enhanced by using organic materials which contain different functional groups. Various amendments are utilized either to mobilize or to immobilize HMs present in soil. Wu et al. (2012) reported that traditional organic materials (rice straw and clover) can be considerably more powerful and ecologically agreeable amendments compared to the chelating agent, ethylenediamine-*N*,*N*'-disuccinic acid (EDDS), in improving phytoremediation efficiency of Cd-tainted soil. Traditional organic amendments can be significantly more compelling and environment friendly than EDDS in enhancing phytoremediation proficiency of soil contaminated with Cd. Wang et al. (2013) reported that, in phytostabilization and phytoextraction of Cd and Pb, organic manure and arbuscular mycorrhizal fungi play a positive synergistic role. These amendments can also increase mobility and leaching of HMs to groundwater by adding soluble organic ligands (Houben et al. 2013). Heavy metal mobility is also affected by the amount of organic matter added in soil.

Phosphate combinations have a wide range of HMs (Loganathan et al. 2008; Wuana and Okieimen 2011). Cadmium contamination of agricultural soils is of explicit worry in light of the fact that frequent utilization of fertilizers (Cd-containing P) is a risk to the food chain and human well-being. Production of P fertilizers is done from the phosphate rocks and most Cd in P fertilizers originates from it. Albeit many nations have defined threshold levels for Cd and different HM concentrations in soils for the utilization of metropolitan biosolids, such limits have not been set up for the use of fertilizers (Bolan et al. 2014).

The foremost rule in the mobilization strategy is to discharge HMs into soil, which is further evacuated by higher plants. Interestingly, in the immobilization method the concerned HMs are expelled from soil solution through either sorption, precipitation, or complexation reactions (Loganathan et al. 2008). Green plants have a huge capacity to take up contaminants from the environment and accomplish their detoxification by different mechanisms as shown in Fig. 3.

5 Conclusion

One of the major challenges faced by the world at present is a significant increase in soil pollution due to organic and inorganic contaminants, especially heavy metals. Research in recent years has shown that phytoremediation by trees can be a green, sustainable, and promising solution to the environmental pollution problems as trees possess physiological properties which suit for the same purpose. Utilization of trees for phytoremediation of heavy metal-contaminated sites is a rapidly emerging technology. Still, only a limited number of studies have been conducted on native tree species to identify them as successful candidates for cleanup of heavy metal-contaminated sites. Hence, the need of the hour is to undertake more well-documented, intensive, and well-designed projects in order to promote the putative candidature of trees for phytoremediation purpose.

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Index

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