Chapter 8 Genetic Diversity and Heavy Metal Stress in Plants

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Abstract Heavy metals are considered as potent pollutants due to their widespread occurrence and their acute and chronic toxic effect on plants, animals, and humans. Variation is of great theoretical importance because it is the raw material on which natural selection acts to influence the evolution of hyperaccumulation. Natural variation is also important basis for the development of hyperaccumulation technology as it indicates the potential for improvement of plant traits through selective breeding, and provides variable genetic markers that can be studied by crossbreeding and molecular techniques. Although some degree of hyperaccumulation occurs in all members of the species that can hyperaccumulate heavy metals, quantitative genetic variation in the ability to hyperaccumulate have been reported, both between and within populations. Genetic diversity and variability analysis have proved to be an effective method in grouping accessions for effective management and utilization in genetic improvement of plants for enhanced phytoextraction. The existing genetic diversity in crops can be used for phytoextraction by identifying easily cultivable, high biomass yielding plants, and practicing selection in future generations.

Keywords Heavy metals **·** Phytoextraction **·** Hyperaccumulation **·** Variability

8.1 Introduction

Stress is an environmental factor that limits crop productivity or causes a reduction in biomass (Grime [1979](#page-26-0); Robert-Seilaniantz et al. [2010](#page-31-0)). Plants are exposed to a variety of stresses in natural environments that may occur singly or concurrently

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(Mittler and Blumwald [2010\)](#page-29-0). Abiotic stress is defined as any environmental condition which reduces the growth, survival, and/or fecundity of plants below optimum levels (Boscaiu et al. [2008;](#page-23-0) Cramer et al. [2011\)](#page-24-0). Abiotic stresses include parameters like temperature, humidity, light intensity, water supply, mineral availability, oxidative stress, and heavy metal toxicity, all of which determine the growth of a plant (Bhargava and Srivastava [2013](#page-23-1)). These stresses adversely affect growth and productivity, and trigger a series of morphological, physiological, biochemical, and molecular changes in plants (Ahmad et al. [2012a,](#page-21-0) [b](#page-21-1); Bhatnagar-Mathur et al. [2008\)](#page-23-2). The stress factors are a menace for plants and prevent them from reaching their full genetic potential and limit crop productivity worldwide (Mahajan and Tuteja [2005\)](#page-28-0). The effect of stresses is more pronounced in plants since the plants being sessile cannot escape from abiotic stress factors and are continuously exposed without any protection. The stress caused by abiotic factors alter plant metabolism leading to negative effects on growth, development, and productivity of plants (Rao et al. [2006](#page-30-0)). It is estimated that environmental stresses limit crop production by more than 50 $\%$ and as much as 70 $\%$ (Boyer [1982](#page-23-3); Wang et al. [2003;](#page-33-0) Mittler [2006](#page-29-1)). If the stress becomes harsh or continues for longer periods it may lead to unbearable metabolic burden on cells, reduced growth and ultimately plant death. Thus, the losses worth hundreds of million dollars each year due to reduction in crop productivity and crop failure as a result of different stresses are threatening the sustainability of agricultural industry. However, plants have developed specific mechanisms that enable them to detect environmental changes and respond to complex stress conditions, minimizing damage while conserving valu-able resources for growth and reproduction (Atkinson and Urwin [2012](#page-22-0)).

8.2 Heavy Metals

Different metals are required by plants in a wide range of concentrations. During the evolution of angiosperms, the metal requirements were strongly steered by the demands of physiological processes in different organelles, cells, tissues, and whole plants (Ernst [2006](#page-25-0)). Heavy metals, the ubiquitous environmental contaminants, are members of an ill-defined group of elements who have a specific gravity of more than 5 $g/cm³$ in their standard state (Padmavathiamma and Loretta [2007;](#page-30-1) Bothe [2011;](#page-23-4) Bhargava and Srivastava [2014](#page-23-5)). According to this criterion, a total of 53 elements are regarded as heavy metals some of which are of importance to living forms while others are toxic. Heavy metals such as iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), cobalt (Co), or molybdenum (Mo) are essential for the growth of life forms while others have a single function such as vanadium (V) in some peroxidases and nitrogenases, or nickel (Ni) in the hydrogenases (Bothe [2011\)](#page-23-4). Heavy metals like cadmium (Cd), lead (Pb), uranium (U), thallium (Tl), chromium (Cr), silver (Ag), and mercury (Hg) are toxic to organisms. Arsenic is a metalloid but is usually classified as a heavy metal. In the soil metals, it may exist in the following forms:

- 1. Bound to organic matter;
- 2. As ions occupying ion exchangeable sites and specifically adsorbed on inorganic soil constituents;
- 3. Free metal ions and soluble metal complexes in solution;
- 4. Precipitated or insoluble forms like oxides, sulfides, carbonates, and hydroxides; and
- 5. Entrapped in the structure of silicate minerals.

8.2.1 Sources of Heavy Metals

Contamination of soil, aqueous streams, and ground water with toxic metals poses a major environmental problem and is a hazard to human health (Bothe [2011;](#page-23-4) Bhargava et al. [2012a\)](#page-23-6). This contamination is primarily due to human activities that have resulted in the increased release of heavy metals in the environment. Heavy metals in atmosphere, soils, water, and sediments pose a serious problem: they can enter and pass through the food chains, and in contrast to organic xenobiotics cannot be degraded by microorganisms. The problems with metal contamination are particularly pronounced in localities where industrial exploitation has led to accumulation of extreme concentrations of these substances, like the surroundings of smelters, tanneries, waste treatment plants, or mining sites (Baldrian and Gabriel [2002\)](#page-22-1). Air emissions from combustion plants, oil, mining, smelting, electroplating, and military and waste practices are the common contributors of heavy metals in the environment (Sharma and Agrawal [2005](#page-31-1); Bhargava et al. [2008,](#page-23-7) [2012a](#page-23-6), [b](#page-23-8); Bhargava and Srivastava [2014\)](#page-23-5).

8.2.2 Importance of Heavy Metals

Metals play a variety of roles in all living organisms. Metals are important for the living forms since they are the active centers of many enzymes. The chemical properties of the metal have been recruited for catalyzing key reactions and for maintaining protein structure. Metals are therefore required in minute amounts for normal cell metabolism, and their intake is subject to intricate homeostatic mechanisms (Bhargava et al. [2012b](#page-23-8)). Metals may act as structural elements, stabilizers of biological structures, components of control mechanisms (e.g., in nerves and muscles), and in particular are activators or components of redox systems. Some of the metals are essential elements, and their deficiency results in impairment of biological functions. An overview of the different uses of heavy metals in plants is provided in Table [8.1.](#page-3-0)

| Metal | Beneficial effects of heavy metals | References |
|----------------|--|---|
| Cu | Important role in $CO2$ assimilation and ATP synthesis Component of plastocyanin and cytochrome oxidase | Thomas et al. (1998) Demirevska-kepova et al. (2004) |
| Fe | Synthesis of chlorophyll Component of cytochromes | Miller et al. (1995), Spiller et al. (1982) Soetan et al. (2010) |
| Z _n | Synthesis of cytochrome Synthesis of tryptophan and auxin Reduce the adverse effects of short periods of heat and salt stress | Tisdale et al. (1984) Alloway (2004), Brennan (2005) Disante et al. (2010), Tavallali et al. (2010) |
| Co | Inhibition of ethylene production Role in salt tolerance | Lau and Yang (1976) Ibrahim et al. (1989) |
| Mn | Activation of enzymes like decarboxy- lating malate dehydrogenase, isocitrate dehydrogenase, and nitrate reductase | Mukhopadhay and Sharma (1991) |
| Mo | Regulatory component in the mainte- nance of nitrogen fixation in legumes Integral part of molybdenum cofactor (Moco) which binds to molybdenum- requiring enzymes | Kaiser et al. (2005), Soetan et al. (2010) Bittner et al. (2001), Mendel and Haensch (2002), Kaiser et al. (2005) |
| Ni | Cofactor of enzymes involved in DNA biosynthesis and amino acid metabolism Component of the enzyme urease | Arinola et al. (2008) Aydinalp and Marinova (2009) |
| Hg | No beneficial effect reported | |

Table 8.1 The importance of heavy metals for plants

8.3 Adverse Effects of Heavy Metals on Plants

Heavy metals are considered as soil pollutants due to their widespread occurrence and their acute and chronic toxic effect on plants grown on such soils (Yadav [2010;](#page-33-1) Manousaki and Kalogerakis [2011\)](#page-28-1). Heavy metals are absorbed through the root systems and are known to induce changes in the plants at morphological, physiological, and molecular levels (Hall [2002](#page-26-1); DalCorso et al. [2013](#page-24-1)). The toxicity in plants varies according to the species, type of metal, its concentration, chemical structure, and edaphic factors (Schützendübel and Polle [2002](#page-31-2); Nagajyoti et al. [2010](#page-29-2)). Heavy metals induce destruction of chlorophyll, necrosis, turgor loss, reduced seed germination, and inhibition of root penetration and plant growth (Foy et al. [1978;](#page-26-2) Kim et al. [2003](#page-27-0); DalCorso et al. [2008;](#page-24-2) Manousaki et al. [2008;](#page-28-2) Shakya et al. [2008](#page-31-3); Aydinalp and Marinova [2009](#page-22-2); Lamb et al. [2010;](#page-27-1) Singh et al. [2013\)](#page-31-4). Heavy metals also influence homeostatic events like water uptake and transport, transpiration, and nutrient metabolism and leads to the deficiency of minerals like Ca, Mg, K, and P (Fodor [2002;](#page-26-3) Poschenrieder and Barceló [2004](#page-30-2)). Table [8.2](#page-4-0) depicts the toxic effects of different heavy metals on plant growth and development. The uptake and accumulation of nutrients is influenced by alteration in the

| Metal | Toxic effect | References |
|-------|--|--|
| Zn | Chlorosis Purplish-red color in leaves Inhibition of ribulose-1,5-bisphosphate- carboxylase/oxygenase (RuBisCO) Growth retardation in roots and shoots | Ebbs and Kochian (1997) Lee et al. (1996) Van Assche and Clijsters (1986) Choi et al. (1996), Ebbs and Kochian (1997) , Fontes and Cox (1998) |
| Hg | Phytotoxicity and physiological disorders in plants Closure of leaf stomata and physical obstruction of water flow in plants | Zhou et al. (2007) Zhang and Tyerman (1999) |
| Cu | Growth retardation and leaf chlorosis Generation of oxidative stress, ROS, disturbance of metabolic pathways and damage to macromolecules | Enyedi et al. (1992), Lewis et al. (2001) Stadtman and Oliver (1991), Hegedus et al. (2001) Messer et al. (2005), Israr and Sahi (2006) , Cargnelutti et al. (2006) |
| Co | Adverse effect on shoot growth and biomass | Li et al. (2009) |
| Mn | Chlorosis, puckering and crinkling of leaves, Leaf abscission, Loss of apical dominance Cytoplasmic injures and plasma membrane rupturing in the outer root cap and meristematic cells | El-Jaoual and Cox (1998), Sirkar and Amin (1974) Santandrea et al. (1998) |
| Pb | Inhibition of enzyme activities, water imbalance, alterations in membrane permeability and disturbs mineral nutrition | Sharma and Dubey (2005) |
| Cr | Inhibition of chlorophyll biosynthesis Inhibition of plant growth, chlorosis in young leaves, nutrient imbalance, wilting of tops, and root injury Induces oxidative stress by increasing the production of ROS | Vajpayee et al. (2000) Chatterjee and Chatterjee (2000), Dixit et al. (2002), Sharma et al. (2003) , Scoccianti et al. (2006) Reddy et al. (2005) |
| Ni | Alteration in the lipid composition and H-ATPase activity of plasma membrane Chlorosis and necrosis Suppression of the hydrolysis of RNA and proteins by inhibiting the activity of ribonuclease (RNase) and protease | Ros et al. (1992) Pandey and Sharma (2002), Rahman et al. (2005) Maheshwari and Dubey (2007) |
| Cd | Inhibition of respiration Inhibition of photosynthesis Inhibition of calmodulin-dependent phosphodiesterase activity | Llamas et al. (2000) Kumar and Kumar (1999) Rivetta et al. (1997) |
| Mo | Inhibits tasseling, anthesis and the development of sporogenous tissues | Agarwala et al. (1978), Martin et al. (1995) |

Table 8.2 Toxic effects of heavy metals on plants

water absorption and solute permeability caused by the heavy metals (Hernández et al. [1997\)](#page-26-5). The accumulation of heavy metals in plants and their subsequent release during decomposition facilitates their recycling in the ecosystem (Kim et al. [2003\)](#page-27-0). This pathway regulates the level of toxic metals in the biosphere.

The response of plants upon exposure to heavy metal stress is primarily due to the generation of reactive oxygen species (ROS). Various metals either generate ROS directly through Haber–Weiss reactions or overproduction of ROS (Schützendübel and Polle [2002](#page-31-2); Mithofer et al. [2004](#page-29-7); Anjum et al. [2012\)](#page-22-5). Thus, the occurrence of oxidative stress in plants could be the indirect consequence of heavy metal toxicity. The possible sequential events of ROS-induced damage development in sensitive plants in response to heavy metal stress are presented in Fig. [8.1](#page-5-0). The indirect mechanisms include their interaction with the antioxidant system (Srivastava et al. [2004](#page-31-14)), disrupting the electron transport chain (Qadir et al. [2004](#page-30-7)), or disturbing the metabolism of essential elements (Dong et al. [2006\)](#page-25-7). Heavy metals also cause membrane damage through various mechanisms like the oxidation of and cross-linking with protein thiols, inhibition of key membrane protein such as H^+ -ATPase, or causing changes in the composition and fluidity of membrane lipids (Meharg [1993\)](#page-28-7). Heavy metals may also impede plant growth indirectly by depriving plants of nutrients required for growth by inhibition of root growth and transpiration, or due to competition by the metal for uptake carriers. The reduction in root growth can limit nutrient uptake due to reduced root area

Fig. 8.1 Possible biochemical and molecular mechanisms of heavy metal-mediated ROS induction and damage to the development of higher plants (Hossain et al. [2012\)](#page-26-8)

available for mineral absorption (Johnson et al. [2011](#page-27-8)). Another deleterious effects induced by heavy metals exposure in plants are lipid peroxidation, which can directly cause biomembrane deterioration and leakage of ions (Boominathan and Doran [2003](#page-23-11)).

8.4 Effect of Heavy Metals on Humans

Contamination of metals in the environment and human diet represents a persistent problem that is a burden on human health (EPA [2012\)](#page-25-8). Humans are exposed to heavy metals in a variety of ways. Exposure generally occurs by ingestion or inhalation. People who live or work in an area near an industrial site which utilizes these metals are more prone to exposure. Similarly, those living near a site where these metals have been improperly disposed are at equal risk to exposure. Widely dispersed metals like mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As) have no beneficial effects in humans (Morais et al. [2012\)](#page-29-8) but are generally considered most toxic to humans and animals. Moreover, no known homeostasis mechanism exists for them (Draghici et al. [2010](#page-25-9); Vieira et al. [2011\)](#page-32-5). Once absorbed in the human body, the heavy metals may induce several deleterious effects varying from irritation to acute to chronic ones. The nature of effects could be toxic (chronic, subchronic, or acute), neurotoxic, mutagenic, teratogenic, or carcinogenic (Richards [2007\)](#page-30-8). In contrast, the essential elements do not produce toxic effects in plants and animals due to the presence of homeostatic mechanisms which regulate their level in the body (Oliveira da Silva et al. [2005\)](#page-29-9). Table [8.3](#page-7-0) provides an overview of the toxic effects of different heavy metals on human beings.

Cadmium (Cd) is one of the most important pollutants in terms of food chain contamination and has no role in human physiology. Cadmium is naturally present in air, soil, sediments, and unpolluted seawater. The element is emitted to air by mines, metal smelters, and industries using cadmium compounds for alloys, batteries, pigments, and in plastics (Harrison [2001\)](#page-26-9). Human exposure to cadmium occurs through the ingestion of contaminated foodstuffs, by the incineration of municipal waste containing plastics and nickel–cadmium batteries and by cigarette smoking (Lewis et al. [1972](#page-27-9); WHO [2004,](#page-33-2) [2006\)](#page-33-3). Cadmium is known to accumulate in the kidney cortex and causes renal tubular dysfunction (Jarup et al. [1998a;](#page-27-10) Barbier et al. [2005;](#page-22-6) Nordberg [2009](#page-29-10)). Subchronic inhalation exposure to Cd leads to pulmonary effects like emphysema, bronchiolitis, and alveolitis, while high exposure leads to cadmium pneumonitis, an obstructive lung disease characterized by chest pain, bloody sputum, and death of lung tissues (Davison et al. [1988;](#page-24-6) Fernandez et al. [1996](#page-25-10); Hendrick [2004](#page-26-10)). Cd exposure also leads to bone defects like osteomalacia, osteoporosis, spontaneous fractures, and skeletal demineralization (McKenna and Chaney [1991;](#page-28-8) Strehlow and Barltrop [1988](#page-32-6); Jarup et al. [1998b;](#page-27-11) Staessen et al. [1999;](#page-31-15) Kazantzis [2004;](#page-27-12) Young [2005](#page-34-2)). Some studies have suggested an association of cadmium and renal cancer in humans (Il'yasova and Schwartz [2005\)](#page-26-11) although later researchers have doubt over these findings.

| Metal | Symptoms | References |
|-------|--|---|
| Zn | Lethargy and focal neurological deficits Metal fume fever | Murphy (1970) Kuschner et al. (1997) |
| | Epilepsy and transient global ischaemia | Weiss et al. (2000) |
| Hg | Sperm immotility High sister chromosome exchanges/cell and induced C-anaphases (abnormal mitosis) | Ernst and Lauritsen 1991 Rao et al. (2001) |
| Cu | Tyrolean cirrhosis Wilson's disease Alzheimer's disease | Muller et al. (1996) Brewer (2001) Brewer (2009, 2012) |
| Co | Abnormal lymphocyte function Hand tremor, incoordination, cognitive decline, depression, vertigo Arrhythmias and cardiomyopathy | Hart et al. (2006), Daou et al. (2011) Tower $(2010a, b)$ |
| Mo | Acute psychosis with visual and auditory hallucinations | Momcilović (1999) |
| Pb | Memory and learning deficits Cognitive and behavioral impairments Chronic lead nephropathy Cancer | Needleman and Landrigan (1981) Devi et al. (2005) Brewster and Perazella (2004) van Wijngaarden (2012) |
| Cr | Dizziness, headache, and weakness Cancer of gastrointestinal tract and central nervous system | ATSDR (2000) Costa and Klein (2006), Zhitkovich (2011) |
| Ni | Nausea, vomiting, abdominal pain, diar- rhea, giddiness Allergic contact dermatitis Nasal and lung cancer | Sunderman et al. (1988) EHC (1991), Cavani (2005) Costa et al. (2005), Lu et al. (2005) |
| Cd | Diabetes mellitus Neurodegeneration; vascular-type dementia (VD) High blood pressure and cardiovascular disease Reduced birth | Edwards and Prozialeck (2009) Mizuno and Kawahara (2013) Telisman et al. (2001) |
| As | Hypertension Anemia and leukopenia Diabetes mellitus | Lee et al. (2003) , Yoshida et al. (2004) Tay and Seah (1975) Walton et al. (2004) |

Table 8.3 Toxic effects of heavy metals on human beings

Lead has been used since centuries for building materials, pigments to glaze ceramics, water pipes, ammunition, glass and crystals, paints, protective coatings, acid storage batteries, gasoline additives, in cosmetics and as a preservative (Florea and Büsselberg [2006](#page-26-12)). However, it is also one of the oldest known and most widely studied occupational and environmental toxins (Gidlow [2004](#page-26-13)). Lead contamination is one of the greatest concerns for human health. Human exposure to lead occurs primarily through drinking water, airborne lead-containing particulates (especially in cigarette smoke and fumes of petroleum products), and leadbased paints. The danger of Pb is more pronounced due to its low mobility even under high precipitation. The half-life of lead in blood is about 1 month and in

the skeleton 20–30 years (WHO [1995\)](#page-33-6). The toxicology of organolead has been extensively reviewed by Grandjean and Nielsen ([1979\)](#page-26-15). Tetraethyllead (TEL) and tetramethyllead (TML) are the main constituents in organolead. Both the tetraethylated or methylated forms are degenerated in the body to the trivalent organic forms, which are highly toxic. The toxicity of organolead differs from inorganic lead compounds depending on alkylation, while the toxic effects of TEL and TML are essentially similar, although the toxicities of these compounds seem to vary by species in animal experiments (Grandjean and Nielsen [1979;](#page-26-15) Florea and Büsselberg [2006\)](#page-26-12). In adults, inorganic lead does not penetrate the blood–brain barrier, whereas this barrier is less developed in children. The children are especially susceptible to lead exposure and subsequent brain damage due to higher permeability of the blood–brain barrier due to which adverse effects of Pb occur at lower threshold levels than in adults. Lead toxicity causes dysfunction of the kidneys, reproductive, and cardiovascular systems; inhibition of hemoglobin synthesis; and damage to the central nervous systems (Kantor [2006;](#page-27-15) Ogwuegbu and Muhanga [2005\)](#page-29-16). Some recent reports have suggested a correlation between lead exposure and carcinogenicity (Siddiqui et al. [2002;](#page-31-16) Xu et al. [2006;](#page-33-7) Rousseau et al. [2007;](#page-31-17) Alatise and Schrauzer [2010](#page-22-7)).

Soil is contaminated with zinc (Zn) emanating from sewage sludge or urban composts, fertilizers, emissions from municipal waste incinerators, residues from metalliferous mining, the metal smelting industry, and other human activities (Yadav [2010](#page-33-1)). Of the 2–3 g Zn in human body, about 90 % of Zn is found in muscles and bones, while prostate, liver, the gastrointestinal tract, kidney, skin, lung, brain, heart, and pancreas also contain estimable concentrations of the metal (Wastney et al. [1986](#page-33-8); Llobet et al. [1988;](#page-28-10) Bentley and Grubb [1991](#page-22-8); He et al. [1991\)](#page-26-16). Zn causes the same signs of illness as does lead and is often mistaken as lead poisoning. Common signs of Zn toxicosis include diarrhea, vomiting, anemia, epigastric pain, and abdominal cramps (Brown et al. [1964;](#page-24-11) Porea et al. [2000;](#page-30-10) Haase et al. [2008\)](#page-26-17).

Arsenic (As), a metalloid, occurs in two oxidation states: a trivalent form, arsenite (As₂O₃; As III), and a pentavalent form, arsenate (As₂O₅; As V) (Ratnaike [2003\)](#page-30-11). Arsenic is often present in plants and animals without any adverse health effect, its toxicity usually depending on the oxidation state and chemical species. The primary route of exposure of inorganic arsenic is through underground drinking water with elevated arsenic concentrations which gradually leads to chronic arsenicosis (Chakraborti et al. [2004](#page-24-12); Bhattacharya et al. [2007](#page-23-16); Mudhoo et al. [2011\)](#page-29-17). Drinking water contaminated with arsenic has been found in both developed and developing countries and is a global health problem affecting millions of people, especially in South Asia (Ahsan et al. [2000](#page-21-4); Mazumder et al. [1998;](#page-28-11) Sun [2004](#page-32-13)). The major source of organic arsenic is mainly fish and seafood, but the organic exposure appears to be much less toxic than the inorganic forms (Uneyama et al. [2007](#page-32-14)). Arsenic is known to form complexes with coenzymes leading to inhibition of production of adenosine triphosphate, the main energy yielding molecule in the body. Arsenic toxicity causes an immune disorder wherein the body's immune system attacks part of its own peripheral nervous system resulting

in muscle weakness. Arsenic is carcinogenic in its oxidation states and high exposure often causes death.

Mercury (Hg) is a unique metal due to its existence in different forms e.g., HgS, Hg^{2+} , Hg° , and methyl-Hg. Hg released to the soil mainly remains in solid phase through adsorption onto sulfides, clay particles, and organic matters. Methylmercury, the common organomercurial species, is of particular concern because of its toxicological characteristics, a long biological half-life and biomagnification through the trophic chain. Mercury is used as a pharmaceutical, in the gold industry, as a component of barometers, thermometers, dental products, electrical equipment, control devices, and in fungicides. The high usage of mercury has resulted in the widespread occurrence of mercury contamination in the entire food chain. The 'Minamata disease,' first reported from Japan in 1956, is the most known incident of organic mercury poisoning which was caused by the release of methylmercury in the industrial wastewater (Weiss [1996\)](#page-33-9). Oral exposure to organomercurial compounds reportedly leads to gastrointestinal and associated disorders like diarrhea, irritation, blisters in the gastrointestinal tract, vomiting, abdominal pain, constipation, and gastritis (Jalili and Abbasi [1961;](#page-27-16) Al-Saleem [1976](#page-22-9); Pfab et al. [1996;](#page-30-12) Castoldi et al. [2003](#page-24-13); Oliveira Da Silva et al. [2005\)](#page-29-9). Exposure to mercury is known to induce genotoxicity (Rao et al. [2001;](#page-30-9) Bonacker et al. [2004\)](#page-23-17) and adversely affect the nervous system (Olivieri et al. [2002;](#page-29-18) Counter and Buchanan [2004](#page-24-14); Johnson [2004\)](#page-27-17), renal system (Ellingsen et al. [2000\)](#page-25-15), reproductive system (Dickman and Leung [1998\)](#page-25-16), immune system (Vimercati et al. [2001;](#page-33-10) Prochazkova et al. [2004](#page-30-13)), and the cardiovascular system (Sorensen and Murata [1999](#page-31-18)).

8.5 Response of Plants to Heavy Metals

Plants are sensitive to heavy metals in a variety of ways that are enumerated below:

- 1. Uptake and accumulation of metals by binding to extracellular exudates and constituents of the cell wall.
- 2. Extrusion of metals from cytoplasm to the extranuclear compartments.
- 3. Complexation of the metal ions inside the cells by complex molecules.
- 4. Concentration of osmolytes and osmoprotectants and induction of enzyme systems.
- 5. Alteration of plant metabolism (Cho et al. [2003\)](#page-24-15).

Baker [\(1981](#page-22-10)) has classified the plants growing on metalliferous soils into three categories:

(i) Excluders—These plants prevent uptake of toxic metals into root cells (de Vos et al. [1991](#page-24-16)). As a result the metal concentrations in the shoot are maintained up to a critical value, at a low level across a wide range of soil concentration.

- (ii) Accumulators—Accumulators do not prevent metals from entering the roots and allow bioaccumulation of high concentration of metals mainly in the aboveground plant parts. For example, members of the order Caryophyllales show a general ability to accumulate metals in their shoot (Broadley et al. [2001\)](#page-24-17).
- (iii) Indicators—In these plants the internal concentration reflects the external levels (McGrath et al. [2002](#page-28-12)).

Hyperaccumulators are a subgroup of accumulator species often endemic to naturally mineralized soils, which accumulate high concentrations of metals in their foliage, while storing lower concentrations in their roots (Reeves and Brooks [1983;](#page-30-14) Brooks [1987](#page-24-18); Baker and Brooks [1989;](#page-22-11) Raskin et al. [1997;](#page-30-15) Macnair [2003\)](#page-28-13). Bioconcentration factor (BCF) is the ratio of metal concentration in the shoot tissue to the soil (McGrath and Zhao [2003\)](#page-28-14). Hyperaccumulators have a BCF greater than 1, sometimes reaching as high as 50–100, while most other plants have metal BCF values of less than 1, which means that it takes longer than a human lifespan to reduce soil contamination by 50 % (Peuke and Rennenberg [2005\)](#page-30-16). Hyperaccumulation of heavy metal ions is a striking phenomenon exhibited by approximately <0.2 % of angiosperms and reported to occur in over 450 species of vascular plants from 45 angiosperm families with most plants belonging to the families Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae (Baker and Whiting [2002;](#page-22-12) Padmavathiamma and Li [2007](#page-29-19); Rascio and Navari-Izzo [2011;](#page-30-17) Bhargava et al. [2012a](#page-23-6)). Metal hyperaccumulators come from a wide range of taxonomic groups and geographic areas, and as such have a wide diversity of morphological, physiological, and ecological characteristics (Pollard et al. [2002\)](#page-30-18).

8.6 Genetic Diversity

Genetic differentiation between individuals is the basis for the evolutionary change of species, populations, and lineages. Biological diversity is defined as the variation present in all species of plants and animals, their genetic material and the ecosystems in which they occur (Rao and Hodgkin [2002](#page-30-19)). Diversity can occur at three levels: genetic diversity (variation in genes and genotypes), species diversity (species richness), and ecosystem diversity (communities of species and their environment). The Rio Convention of 1993 on biodiversity has also recognized 3 levels of biological variability viz. (i) diversity of ecosystems and landscapes, (ii) species richness, and (iii) genetic variation within species. Genetic diversity is the amount of genetic variability among individuals of a variety, or population of a particular species (Brown [1983](#page-24-19)). It forms the basis for survival and adaptation, and makes it possible to advance the adaptive processes on which evolutionary success depends (Rao and Hodgkin [2002\)](#page-30-19). Study of genetic diversity is the process by which variation among individuals or groups of individuals or populations

is analyzed by a specific method or a combination of methods (Mohammadi and Prasanna [2003](#page-29-20)). Assessment of genetic diversity is invaluable in genetic improvement of plants as it helps in the identification of diverse parental combinations to create segregating progenies with maximum genetic variability and facilitates introgression of desirable genes from diverse germplasm into the available genetic base (Barrett and Kidwell [1998](#page-22-13); Thompson et al. [1998;](#page-32-15) Bhargava et al. [2007](#page-23-18), [2008;](#page-23-7) Fuentes and Bhargava [2011\)](#page-26-18). Many tools are now available for studying variability and the relationships among accessions that include seed protein electrophoresis, isozymes, and various types of molecular markers.

8.7 Genetic Diversity and Heavy Metals

Genetic diversity is a prerequisite for adaptive evolution. Variation is of great theoretical importance, because it is the raw material on which natural selection acts to influence the evolution of hyperaccumulation (Pollard et al. [2002](#page-30-18)). The relationship between hyperaccumulation and tolerance can be easily understood by studying the patterns of variation in these types. Natural variation is also important basis for the development of hyperaccumulation technology as it indicates the potential for improvement of plant traits through selective breeding, and provides variable genetic markers that can be studied by crossbreeding and molecular techniques (Pollard et al. [2002\)](#page-30-18). Although some degree of hyperaccumulation occurs in all members of the species that can hyperaccumulate heavy metals, quantitative genetic variation in the ability to hyperaccumulate have been reported, both between (Pollard and Baker [1996;](#page-30-20) Bert et al. [2000](#page-23-19); Escarré et al. [2000;](#page-25-17) Pollard et al. [2002;](#page-30-18) Assunção et al. [2003](#page-22-14); Roosens et al. [2003](#page-31-19)) and within populations (Pollard and Baker [1996;](#page-30-20) Meerts and van Isacker [1997;](#page-28-15) Escarré et al. [2000;](#page-25-17) Pollard et al. [2002\)](#page-30-18). Such variation does not appear to correlate positively with either the concentration of heavy metals in the soil or the degree of metal tolerance in the plants.

The existence of genetic difference in heavy metal uptake and accumulation, as well as tolerance has been found in diverse crop plants. Rice is one of the most utilized cereals for edible purposes in different parts of the world. In rice, several reports are available that show enormous variation for heavy metal tolerance (Aniol and Gustafson [1990;](#page-22-15) Yang et al. [2000;](#page-33-11) Zhang et al. [2000;](#page-34-5) Arao and Ae [2003](#page-22-16); Liu et al. [2003\)](#page-28-16). In fact, Cheng et al. [\(2006](#page-24-20)) found significant genotypic variation for Cd, Cr, As, Ni, and Pb in the grains while investigating nine rice genotypes grown in six locations for two successive years. A comparative study on cadmium uptake by several rice cultivars was carried out by Morishita et al. [\(1987](#page-29-21)) in Andisols with a low-total cadmium concentration in soil. It was observed that japonica brown rice varieties had the lowest average uptake rate compared to the other three varieties namely, javanica, indica, and Hybrid. Average cadmium levels in brown rice ranged from 2.1 to 27.0 mcg kg−1 among 28 japonica varieties and from 4.1 to 55.5 mcg kg⁻¹ among 23 indica varieties. Arao and Ishikawa [\(2006](#page-22-17)) reported that 49 varieties of rice were cultivated in Cd-polluted soils; the japonica

varieties were categorized into the low grain Cd group. Several indica or indica– japonica varieties accumulated considerable amounts of Cd in grains as well as in straw (Arao and Ishikawa [2006](#page-22-17)). Liu et al. ([2003\)](#page-28-16) conducted a study on 20 rice cultivars of different genotypes and origins on cadmium supplemented soils. The result showed that the effects of Cd on rice growth and development varied greatly among cultivars. Some varieties were highly tolerant to soil stress imposed by cadmium, while others were very sensitive. Differences existed among the cultivars for Cd uptake and distribution of rice plants (Liu et al. [2003](#page-28-16)). Liu et al. [\(2007](#page-28-17)) conducted pot soil experiments with two rice cultivars at different levels of Cd to understand certain mechanisms causing the variations between rice cultivars with regard to Cd uptake and accumulation. The results showed that the rice cultivar with higher concentrations of LMWOA (low-molecular-weight organic acids) in soil accumulated more Cd in the plants. The results indicated that LMWOA secretion by rice root, especially in Cd-contaminated soils, was likely to be one of the mechanisms determining the plant Cd uptake properties of rice cultivars (Liu et al. [2007\)](#page-28-17).

In *Brassica juncea*, high variability between species and between cultivars within a species for the accumulation of heavy metals has been documented by Salt et al. ([1995\)](#page-31-20). Kastori et al. ([2010\)](#page-27-18) observed high genetic variability between populations of wild sunflower species and hybrids in the uptake and tissue concentration of heavy metals. Coefficient of variation of concentration of nonessential microelements in wild populations varied from 7.7 to 73.8. The average coefficient of variation was the highest for Cr, Ni, and Zn in hybrids and for Cd, Ni, and Cr in wild species.

Genetic diversity for heavy metal accumulation has been reported in underutilized crops like *Chenopodium* and *Amaranthus* (Shukla et al. [2006](#page-31-21); Bhargava et al. [2008,](#page-23-7) [2010](#page-23-20)). In chenopods, significant genotypic differences have been reported in the heavy metal uptake by plants both at inter and intraspecific level (Bhargava et al. [2008\)](#page-23-7). The study was undertaken to characterize and classify the qualitative variation among the chenopod germplasm based on mineral composition of the foliage for 10 minerals (Table [8.4](#page-13-0)). The analysis of variance exhibited significant differences for all the 10 minerals under study (data not shown) indicating the presence of large amount of variation for different minerals among the accessions. Principal component analysis (PCA) showed that the first 4 PCs (Principal Component) accounted for 74.70 $%$ of the total variance among the accessions (Table [8.5](#page-16-0)). The first PC (PC1) accounted for 41.96 % of the total qualitative variation and had nickel, zinc, and chromium with high positive and copper with high-negative coefficients. Cluster analysis grouped the accessions into 4 major clusters. The first cluster, which showed maximum diversity, had 17 accessions, all of *Chenopodium quinoa* having high content of most of the heavy metals viz. zinc, chromium, nickel, and cadmium. Cluster II was the largest consisting of 18 accessions which had low content of nickel, cadmium, and chromium. Cluster III contained three accessions that had lowest amount of calcium, iron, magnesium, and zinc, while accessions in cluster IV were characterized by high levels of calcium, sodium, magnesium, nickel, chromium, and cadmium.

(continued)

Table 8.4 (continued) **Table 8.4** (continued)

Extensive variation for cadmium tolerance and accumulation has also been observed among populations of the partridge pea (*Chamaecrista fasciculata*), a leguminous pioneer species native to the eastern United States (Henson et al. [2013\)](#page-26-19). At the germination stage, *C. fasciculata* did not exhibit between-population variation for tolerance. However, between-population variation for tolerance was noted in plant growth, as reflected by their tolerance indices. *C. fasciculata* accumulated cadmium throughout all plant parts specifically noted for their role in interspecific interactions: stems, leaves, pollen, seeds, and root nodules. It was concluded that the potential of *C. fasciculata* for use in remediation or restoration varied significantly across populations, demonstrating the importance of considering seed source when screening populations of *C. fasciculata* for utilization in phytoremediation (Henson et al. [2013](#page-26-19)).

Variability for heavy metal tolerance has also been reported extensively in tree species like willows (*Salix* sp.). Willows have shown significant variations in tolerance across species, varieties, and clones. Significant variations in metal tolerance were found among willow species and clones exposed to cadmium, copper, or arsenic (Punshon and Dickinson [1999;](#page-30-21) Kuzovkina et al. [2004](#page-27-19); Purdy and Smart [2008;](#page-30-22) Magdziak et al. [2011\)](#page-28-18). Numerous studies have indicated high capacity for cadmium and zinc uptake in *Salix integra* (Yang and Chen [2008;](#page-33-12) Harada et al. [2010;](#page-26-20) Liu et al. [2011\)](#page-28-19). Wang et al. [\(2014](#page-33-13)) examined the variations in lead (Pb) tolerance and accumulation of three cultivated varieties of *S. integra*, a shrub willow native to northeastern China, using hydroponic culture in a greenhouse. The tolerance and accumulation of Pb varied among the three willow varieties depending on the Pb concentration. All three varieties had a high-tolerance index (TI) and EC50 value but a low-translocation factor (TF), indicating that Pb sequestration is mainly restricted in the roots of *S. integra*. Among the three varieties, Dahogntou was more sensitive to the increased Pb concentration than the other two varieties,

| Plant species | Heavy metal | References | | | |
|---------------------------------|--------------------|---------------------------------------|--|--|--|
| Helianthus annus | C _d | Li et al. (1995), (1997) | | | |
| Populus nigra | Cd | Gaudet et al. (2011) | | | |
| Averrhoa carambola | C _d | Dai et al. (2011) | | | |
| Thlaspi caerulescens | Cd, Zn | Zha et al. (2004) | | | |
| | Ni, Zn | Richau and Schat (2009) | | | |
| Thlaspi pindicum | Ni, Zn | Taylor and Macnair (2006) | | | |
| Ipomoea aquatica | Cd | Wang et al. (2009) | | | |
| Dianthus carthusianorum | Zn, Pb | Wójcik et al. (2013) | | | |
| Chenopodium quinoa | C _d | Bhargava et al. (2008) | | | |
| Chenopodium giganteum | Cd, Zn, Ni | Bhargava et al. (2008) | | | |
| $C.$ album | Mg, Zn, Cd | Bhargava et al. (2008) | | | |
| Oryza sativa | Cd | Liu et al. (2005), Wang et al. (2011) | | | |
| Triticum aestivum | Cd | Stolt et al. (2006) | | | |
| Triticum turgidum L. var. durum | Cd | Li et al. (1997) | | | |
| Linum usitatissimum | Cd | Li et al. (1997) , Hocking and | | | |
| | | McLaughlin (2000) | | | |
| Brassica juncea | Ni | Ansari et al. (2015) | | | |
| | Cr | Diwan et al. (2008) | | | |
| Brassica rapa | Zn, Fe, Mn | Wu et al. (2007) | | | |
| Brassica oleracea | Zn, Fe | Kopsell et al. (2004) | | | |
| Apium graveolens | Cd, Pb | Zhang et al. (2013) | | | |
| Amaranthus tricolor | Zn, Fe, Ni, Mn | Shukla et al. (2006) | | | |
| Arabidopsis thaliana | Cu | Kobayashi et al. (2008) | | | |
| | Co, Ni, Cu, Cd, Mo | Baxter et al. (2008, 2012) | | | |
| Pteris vittata | Zn, Cd | Wu et al. (2009) | | | |
| | | | | | |

Table 8.6 Variability for heavy metal tolerance/accumulation in diverse plant species

with the lowest EC50 and TI for root and above-ground tissues. The three varieties revealed various toxicity symptoms of leaf wilting, chlorosis, and inhibition of shoot and root growth under the higher Pb concentrations.

Table [8.6](#page-17-0) depicts the variability for heavy metal accumulation and tolerance reported in diverse plant species.

8.8 Implications of Heavy Metals on Genetic Diversity

The genetic composition of natural populations is constantly modified by natural events (Ungherese et al. [2010\)](#page-32-16). Anthropogenic impact of pollutants can cause severe alterations in the genetic structure of populations. Therefore, the effect of pollutants on genetic variability is fundamental in preserving the evolutionary potential of natural populations. Among the various groups of contaminants, heavy metals seem to strongly affect genetic variability, both directly (via germ

cell mutations) and indirectly (via somatic mutations or ecological and physiological effects) (Bickham et al. [2000](#page-23-21); Belfiore and Anderson [2001;](#page-22-21) De Wolf et al. [2004\)](#page-25-19). Heavy metal exposure can alter the genetic composition of a population by favoring more tolerant genotypes and causing demographic bottlenecks leading to a decrease of genetic variability known as 'genetic erosion' (Van Straalen and Timmermans [2002;](#page-32-19) Ribeiro et al. [2012](#page-30-24); Ribeiro and Lopes [2013\)](#page-30-25). In genetic erosion, small populations become increasingly subject to genetic drift and inbreeding, resulting in loss of genetic variation and a decrease in fitness. Genetic drift will cause allele frequencies to fluctuate, which over time leads to random loss and fixation of alleles and an increase in homozygosity (Bijlsma and Loeschcke [2011\)](#page-23-22). A special case of genetic drift is population bottleneck which occurs when the size of a population is significantly reduced leaving a small collection of genotypes as founders for recovery and expansion (van Straalen and Timmermans [2002](#page-32-19)). Some recent studies have pointed toward an increase in the genetic diversity in metal-polluted environments and a possible role in evolution. In polluted environments, intra- and interpopulation changes at the molecular level proceed rapidly and lead to the formation of new ecotypes in a relatively short time (Słomka et al. [2011\)](#page-31-22). A recent study used ISSR PCR fingerprinting data to analyze the genetic diversity and genetic structure of seven populations of *Viola tricolor*: four growing on soil contaminated with heavy metals (Zn, Pb, and Cd; waste heaps) and three from control soil (Słomka et al. [2011](#page-31-22)). The populations from the polluted sites showed higher genetic polymorphism (%(poly) = 84 %) and gene diversity (H(T) = 0.1709) than the control populations (%(poly) = 75 % and H(T) = 0.1448). The number of private markers detected within metallicolous (MET) populations was more than double that found within nonmetallicolous (NON) populations (15 vs. 7). The STRUCTURE and UPGMA analyses showed clear genetic differences between the NON and MET populations. Based on broad analyses of the genetic parameters, it was concluded that the effect of these polluted environments on the genetic diversity of the MET populations, separating them from the NON populations, is evidence of microevolutionary processes at species level, leading to species divergence and the emergence of local ecotypes better adapted to their different environments.

Sites contaminated by heavy metals (metalliferous sites) are places where microevolutionary processes accelerate due to colonization of the contaminated sites by plants that have a small genome size and have evolved an r-life strategy with the crucial ability to reproduce quickly, owing to fast flowering, seed ripening, and much greater flower and seed yields (Wierzbicka and Rostan´ski [2002;](#page-33-19) Grzes´ [2007](#page-26-23); Vidic et al. [2009\)](#page-32-20). The toxicity of metal pollution can affect the genetic diversity of exposed populations through various means like plant survivorship, recruitment, reproductive success, mutation rates, and migration (Anderson et al. [1994;](#page-22-22) Bickham and Smolen [1994](#page-23-23); Fox [1995;](#page-26-24) Deng et al. [2007\)](#page-25-20). The populations of plants growing at heavy metal contaminated sites are often genetically distinct from the populations of the same species in uncontaminated locations (Assunção et al. [2003;](#page-22-14) Dubois et al. [2003](#page-25-21)). However, conflicting results

have been obtained when genetic variation has been studied among metal-tolerant and nonmetal-tolerant populations. The genetic diversity of the uncontaminated population was found to be similar to that of the contaminated population in *Silene paradoxa* (Mengoni et al. [2000](#page-29-22)), *Agrostis stolonifera* (Wu et al. [1975](#page-33-20)) and *Arrhenatherum elatius* (Ducousso et al. [1990\)](#page-25-22). On the contrary, the reduction of genetic diversity was found in some species like *Deschampsia cespitosa* (Bush and Barrett [1993](#page-24-22)) and *Armeria maritima* (Vekemans and Lefèbvre [1997](#page-32-21)).

Deng et al. ([2007\)](#page-25-20) undertook a detailed study to assess the impact of heavy metal contamination on genetic variation of *Sedum alfredii*, a fleshy perennial herb. *S. alfredii* has been reported to be a Pb accumulator (He et al. [2002\)](#page-26-25) and hyperaccumulator for Zn and Cd (Yang et al. [2002,](#page-33-21) [2004\)](#page-34-8). The genetic diversity and population structure of seven populations of *S. alfredii* growing in lead/zinc (Pb/Zn) mine spoils or in uncontaminated soils were investigated using random amplified polymorphic DNA (RAPD) technology. A significant reduction of genetic diversity was detected in the mining populations. Analysis of molecular variance (AMOVA) and the unweighted pair group method with arithmetic mean (UPGMA) tree derived from genetic distances further corroborated that the genetic differentiation between mine populations and uncontaminated populations was significant (Deng et al. [2007](#page-25-20)) (Fig. [8.2\)](#page-20-0). Reduction in genetic diversity of a mine population was theoretically expected because of the strong bottleneck as a result of strong selection pressure on plants due to heavy concentration of Zn, Cd, and Pb (Bradshaw [1984](#page-23-24); Lefèbvre and Vernet [1990\)](#page-27-22). The reduction of genetic diversity might be caused by a bottleneck effect which preserved the tolerant individuals and decreased the number of sensitive ones (Bickham et al. [2000](#page-23-21)).

Babst-Kostecka et al. ([2014\)](#page-22-23) investigated the genetic variability of *Biscutella laevigata* L. (Brassicaceae), a perennial, strictly outcrossing species, among all 16 known low and high elevation provenances from locations in southern Poland using nine microsatellite markers to assess historical and evolutionary processes shaping its genetic structure. Populations clustered into two groups which corresponded to their edaphic origin and diverged 1200 generations ago. The authors detected a significant decrease in genetic diversity and evidence for a recent bottleneck in metallicolous populations. Environmental conditions, especially the metal concentrations in the soil, appeared to more strongly influence the genetic structure rather than geographic distance (Babst-Kostecka et al. [2014](#page-22-23)). A significant reduction in the genetic diversity (founder and bottleneck effects) in metallicolous compared to nonmetallicolous populations was associated with the colonization of polluted sites and/or evolution of metallicolous populations. As a consequence, populations from natural and anthropogenic locations have adapted to different environmental conditions and have genetically diverged.

Fig. 8.2 The UPGMA tree plot of genetic distance among *S. alfredii* populations grown on metal contaminated soils based on RAPD analysis (Deng et al. [2007\)](#page-25-20)

8.9 Implications of Genetic Diversity/Variability for Phytoremediation

Most of the known hyperaccumulators are small, slow growing, and often are rare species of limited population size and restricted distributions. If desirable traits can be identified in natural hyperaccumulator plants, they could be selected either by conventional breeding techniques, or using new methods of hybridization such as protoplast fusion, or by the manipulation of gene expression in transgenic plants. The ideal phytoremediation plant should combine rapid growth and high biomass along with high metal accumulation in the shoot tissues (Chaney et al. [2000](#page-24-23); Lasat [2002;](#page-27-23) McGrath et al. [2002](#page-28-12)). Thus, understanding the genetic mechanism of metal accumulation in hyperaccumulator species is important because it facilitates the use of various approaches to genetic improvement of plants for metal uptake (Bhargava et al. [2012a\)](#page-23-6). Efficient management and utilization of germplasm requires detailed knowledge of the genetic diversity of agronomic traits for proper characterization of populations to facilitate efficient synthesis of breeding populations that are designed to accomplish specific objectives (Bhargava et al. [2007](#page-23-18), [2008\)](#page-23-7). From the viewpoint of a breeder, the presence of sufficient genetic variability in the base population is a prerequisite for any crop-breeding program. The characters of economic importance are generally quantitative in nature and exhibit a considerable degree of interaction with the environment. Thus, it becomes necessary to compute variability present in the breeding material and its partitioning into genotypic, phenotypic, and environmental ones. The available and potential qualitative variability is interesting for potential users of the germplasm in relation to prospect of isolating different genotypes for phytoextraction of heavy metals. Genetic diversity and variability analysis have proved to be an effective method in grouping accessions for effective management and utilization in genetic improvement of plants for enhanced phytoextraction. The existing genetic diversity in crops can be used for phytoextraction by identifying easily cultivable, high biomass yielding plants, and practicing selection in future generations (Bhargava et al. [2012a\)](#page-23-6).

8.10 Conclusions

Since the mineral homeostasis in plants is under complex genetic control, there is likely to be substantial genetic variation for this control, which opens new avenues for the improvement of mineral accumulation and tolerance by classical breeding or genetic engineering approaches. The available and potential qualitative variability may be of immense interest for potential users of the germplasm in relation to prospect of isolating different genotypes for effective phytoextraction.

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