Chapter 3
Phytoremediation of Zinc-Contaminated Soil and Zinc-Biofortification for Human Nutrition

Li Zhao, Linxi Yuan, Zhangmin Wang, Tianyu Lei and Xuebin Yin

Abstract Phytoremediation of Zinc (Zn) contaminated soil is to remove Zn from soil using Zn-tolerant plants or hyperaccumulators with the assistance of agricultural or biological technologies. Zn biofortification is to increase the natural content of Zn with high bioavailability in staple food crops to provide Zn supplement sources for humans. Researches on understanding the physiological mechanisms of Zn uptake, distribution, storage, and metabolism by plants indicate that the two applications share certain limiting physiological processes. In this chapter, the physiological processes of Zn in plants are introduced and certain regulatory mechanisms are reviewed. Issues related to existing strategies, bottlenecks, and potential improvements on Zn-phytoremediation and Zn-biofortification will also be discussed. Though much remains to be elucidated, the combination of high efficiency of Zn accumulation in plants for phytoremediation and favorable Zn-accumulation in the edible part of staple crops for biofortification appear to be a worthwhile and promising attempt.

Keywords Zinc · Zinc phytoremediation · Zinc biofortification · Physiological processes

---

L. Zhao · L. Yuan · Z. Wang · T. Lei · X. Yin (✉)
Advanced Laboratory for Selenium and Human Health, Suzhou Institute for Advanced Study, University of Science and Technology of China,
Suzhou 215123, Jiangsu, China
e-mail: xbyin@ustc.edu.cn

X. Yin
School of Earth and Space Science, University of Science and Technology of China (USTC), Hefei 230026, Anhui, China

X. Yin and L. Yuan (eds.), *Phytoremediation and Biofortification*,
SpringerBriefs in Green Chemistry for Sustainability,
DOI: 10.1007/978-94-007-1439-7_3,

3.1 Zinc: An Overview

Zinc (Zn) is the twenty-fourth most abundant element on the Earth's crust with a content of 75 mg kg^{-1}. Soil contains 5–770 mg kg^{-1} of Zn with an average of 64 mg kg^{-1}, seawater has 30 µg Zn L^{-1}, and the atmosphere contains 0.1–4 µg Zn m^{-3} (Emsley 2001). Zn in the environment mainly exists in the state of Zn-sulfide and Zn-oxides, and easily associates with many other elements, such as lead (Pb), copper (Cu), cadmium (Cd) to form mineral associations. Zn is a transition metal with atomic number of 30 and has five stable isotopes: ^{64}Zn (48.63 %), ^{66}Zn (27.90 %), ^{67}Zn (4.90 %), ^{68}Zn (18.75 %), and ^{70}Zn (0.62 %). ^{65}Zn is radioactive, with a half-life time of 244.26 days, and it is frequently used as a Zn radiotracer in plants. Zn commonly presents in oxidation states of +1 or +2 in the environment (Brady et al. 1983). Zn^{2+} participates in biological or chemical reactions. In solution, Zn exists in the +2 oxidation state and is redox-stable under physiological conditions for a complete d-shell of electrons (Broadley 2007). Zn tends to form strong covalent bonds with O-, N-, and S- donors and thus forms numerous stable complexes (Greenwood and Earnshaw 1997). Tetracoordinated and hexacoordinated Zn complexes are the most common types, though penta-coordinated complexes also exist (Holleman et al. 1985).

Zn is an essential trace element for plants (Broadley et al. 2007), animals (Prasad 2008), and microorganisms (Sugarman 1983). It is required in a large number of proteins in organisms and is the only metal presenting in all classes of enzymes. In proteins, Zn ions are often coordinated to the side chains of amino acids, such as cysteine and histidine, aspartic acid, and glutamic acid. In organisms, three main functional sites of Zn are recognized, and the function varies with the geometry and characteristics of Zn^{2+}-ligand bonding: structural, catalytic, and cocatalytic (Auld 2001; Maret 2005). Since Zn has no oxidant properties like iron and copper, it exists almost entirely in divalent state, making it easily incorporated into the biological system and safely transports both extra- and intracellularly (Hambidge and Krebs 2007).

Zn is responsible for the normal expression of more than 20 physiological functions in organisms, including immune function, protein synthesis, wound healing, DNA synthesis, and cell division. A large number of proteins in biological systems need Zn to maintain their structural stability and transcription factors. Protection against infections and diseases is related to gene regulation and expression under stress conditions in which Zn is required (Prasad 2010). Zn is also a critical element required for detoxification of highly aggressive free radicals and for structural and functional integrity of biological membranes (Cakmak 2000). Zn supports normal growth and development during pregnancy, childhood, and adolescence. It is also required for proper sense of taste and smell. Zn deficiency is among the major malnutrition in humans and has led to severe diseases, cellular disturbances and impairments, and even large mortality especially in infants and young children.

Although Zn is an essential element for life, excess Zn can be harmful and can cause Zn toxicity to organisms. The free Zn^{2+} in solution is highly toxic to plants, invertebrates, and even some vertebrate species. Acute adverse effects of high Zn intake include nausea, vomiting, loss of appetite, abdominal cramps, diarrhea, and headaches. Long-term exposure of Zn can cause chronic Zn poisoning which will cause a greatly decreased blood copper concentration, anemia, leukocyte rare disease, immunity damaged, weight loss, and other symptoms. Low concentrations of Zn are necessary for normal plant growth; however, a strong phytotoxicity of Zn and retard plant growth can appear with the presence of Zn at over 400 mg/kg. Sewage irrigating crops, especially wheat, with sewage water containing high levels of Zn, will result in uneven seedling emergence, less tiller, plant dwarf, and leaf chlorosis. The diversity of microbes in the Zn-contaminated soil also appears to decrease with increasing Zn content (Moffett et al. 2003).

3.2 Physiological Processes of Zinc in Plants

Zn plays a central role in healthy plant metabolism and growth processes. It is transported in xylem after being absorbed by the roots and then distributed in plant tissues. Zn is unevenly distributed in plants, and Zn concentrations in tissues could differ greatly among plant species. Zn exists in both soluble and insoluble forms in plant tissues. Carboxylic acids, amino acids, phytochelatins (PCs), nicotianamine (NA), and proteins are the main complex organic compounds with soluble Zn. Zn is also found as inorganic Zn-phosphate salts and organic Zn-phytates (White and Broadley 2011).

Concentrations of Zn in plants vary from 25 to 150 mg kg^{-1} DW. The foliar Zn deficiency symptoms appear with a Zn concentration of <15 mg kg^{-1} DW in leaf, while toxicity symptoms become visible when leaf Zn is over 300 mg kg^{-1} DW (Broadley et al. 2007). Detrimental effects of Zn on plant can be observed if plant Zn concentration reaches 400 mg kg^{-1}. Nevertheless, the Zn concentration in the aerial parts can reach up to 1,000 mg kg^{-1} DW for Zn-tolerant plants and even more than 10,000 mg kg^{-1} DW for Zn-hyperaccumulators (Baker and Walker 1990).

As one of the most important micromineral elements, the primary physiological functions of Zn in plant biochemical activities include: (1) activating agent for vitamins; (2) participating in plant cell respiration, promoting photosynthesis via enhancing the production of chlorophyll; (3) catalyzing redox action and accelerating protein oxidation; (4) participating in the synthesis of growth hormones auxin in plants, and (5) promoting plants to thrive with high resistance for diseases and cold. Several physiological processes involved in Zn absorption, transport, and storage by plants are introduced in detail in the following sections.

3.2.1 Zinc Uptake from Soil by Plants

Generally, Zn can be absorbed via roots primarily as Zn^{2+} and/or $Zn(OH)_2$ at high pH in the soil solution. Zn is transported either symplastically or apoplastically after being taken up through root cells (White et al. 2002; Broadley et al. 2007). The uptake of Zn by plants from soil to plant roots is proposed to be driven by the negative electrical potential in plasma membrane and mediated by the complicated metal transport systems on the plasma membrane of root cell. The availability of Zn in the soil to plant, rhizospheric process, and cell membranes transport are thought to be the important biological processes controlling Zn uptake.

The availability of Zn in soil is controlled by the factors that affect the amount of available Zn in soil solution or its sorption–desorption from/into the soil solution. These factors usually include: the total Zn content, chemical forms of Zn compounds, soil properties (organic matter content, carbonate, or phosphate content, granularity, pH), environmental conditions (temperature and humidity), concentrations of other trace elements, and relative biological activities. At low soil pH (<6) the bioavailability of Zn generally increases with increasing replacement of Zn^{2+} by H^+ (Pilon-Smits 2005). A higher temperature can accelerate the biochemical activities in general, thus Zn absorption and relocation processes could be accelerated. Organic matter can either increase the Zn availability in the soil with the formation of soluble organic zinc complexes which are probably capable of absorption into plant roots or decrease its bioavailability by the formation of stable solid-state organic Zn complexes (Alloway 2008).

The rhizosphere process involves complex interactions between plant roots and rhizosphere microbes. Plant roots release a variety of organic compounds (such as organic acid, siderophores, and phenolics) that are the natural carbon resources for microbes (Bowen and Rovira 1991). At the same time, microbes in the rhizosphere stimulate root growth and enhance water and micronutrient absorption (Kapulnik 1996). There are many studies about the roles of rhizosphere microbes in protecting plant from excessive heavy metal toxicity by reducing metal absorption (Delorme et al. 2001; Whiting et al. 2001; Farinati et al. 2009). For example, under the circumstance of excessive Zn, arbuscular mycorrhiza fungi (AMF) can improve plant growth, reduce the Zn toxicity to plants, influence the absorption and translocation of Zn, and facilitate the extraction of Zn by plants from soil (Chen et al. 2003). The secretion from plants and microbes has both positive and negative effects on plant metal uptake: the secretion protects plants by reducing the absorption of metals, or promotes metal absorption by chelating metals to increase metal bioavailability.

Transport of bioavailable ions across the plasma membrane of roots is a critical step in metal uptake and accumulation. Taking Zn^{2+} for example, the absorption dynamics of Zn for plants can be distinguished into two stages. At the very beginning, it is a fast and linear dynamic phase which is related to the Zn^{2+} adsorption on the root cell wall. Then, there is a slower saturated adsorption stage which is related to the transport of Zn^{2+} through the plasma membrane of root cells.

The Zn influx to the cytoplasm of root cells is mainly mediated by various classes of protein transporters on the plasma membrane, though some plasma membrane Ca^{2+} channels are also contributing to Zn^{2+} uptake (White and Broadley 2011).

Some metal transporters of the ZIP protein family are considered to be the predominant uptake systems for Zn in plants (Grotz et al. 1998; Guerinot 2000). The ZIP transporters were characterized in *Arabidopsis* (Grotz et al. 1998), soybean (Moreau et al. 2002), and rice (Ishimaru et al. 2005; Ramesh et al. 2004). The ZIP family includes a set of transport proteins, and these transport proteins have an important feature, i.e., they all can transport Zn^{2+} and other metal ions from the extracellular or organelles lumen into the cytosol (Saier 1999). ZRT1 and ZRT2 are the earliest achieved ZIP family members by gene cloning. They stand for, respectively, high affinity and low affinity of the Zn^{2+} absorption transporters (Zhao and Eide 1996a, b). ZRT1 and ZRT2 are responsible for the absorption of Zn^{2+} across the plasma membrane, while ZRT3 in the vacuole membrane is responsible for shipping Zn^{2+} from the vacuole back to the cytoplasm (Macdiarmidc et al. 2000). IRT1 and IRT2, the main Fe^{2+} uptake systems in *Arabidopsis thaliana* root cells, are found to contribute significantly to the uptake of Zn and Cd by plants. An increase of IRT1 transcript and IRT1 protein levels in the root appears after the treatment of Fe limitation, which in turn leads to IRT1-dependent Cd and Zn accumulation in the roots (Palmgren et al. 2008). In addition to the ZIP transport system, yellow strip-like (YSL) proteins, which have been found mainly involved in Fe transport, are also evaluated to contribute to the uptake of Zn complexed with phytosiderophores or NA (Schaaf et al. 2005). HMA2 and HMA4 are two out of the eight gene encoding members of the type1B heavy metal–transporting subfamily of the *P*-type ATPases in *Arabidopsis thaliana* (Grotz and Guerinot 2006). They are observed to play a primary role in essential Zn homeostasis.

These transport systems, to a great extension, determine the specificity and direction of Zn^{2+} transport, and the concentration of transport protein will decide the speed of Zn^{2+} cross-membrane transport and the accumulation of Zn^{2+} within the membrane. In Zn hyperaccumulator, *Thlaspi caerulescens*, the constitutive expression of a Zn transporter in the root cell membrane is proved to be one of the underlying mechanisms of natural hyperaccumulation. Researches on the molecular mechanism involved in Zn hyperaccumulation and hypertolerance are undergoing. Though much remains to be investigated, the existing knowledge demonstrates that the transmembrane process is one of the key factors that control essential Zn homeostasis (Clemens et al. 2002; Hussain et al. 2004).

3.2.2 Zinc Chelation and Compartmentation in Roots

After the transmembrane process, most of the Zn (primary Zn^{2+}) is chelated by several metal chelators in the cytosol or sequestrated in the vacuoles, keeping a vanishingly low concentration of free Zn ion in the cytosol. This mechanism is the main way of heavy metal detoxification in plants, contributing to metal tolerance and metal hyperaccumulating of plants. A lot of chelating materials have already been discovered for plants and metallothioneins (MTs), PCs, low-molecular chelating agents (LCs) are three typical groups of those metal chelators.

(1) MTs
   MT is a kind of low molecular and cysteine-rich polypeptides found in the cytoplasm of plant cells. The hydrosulfuryl of cysteine residues can detoxify heavy metals (like Zn and Cd) by forming non-toxic or low-toxic complex (Nathalieal et al. 2001). The first MT extracted from plants is wheat EC (Early Cys) protein. It was extracted from mature embryo of wheat, and can combine with Zn^{2+} (Lane et al. 1987). Since then, more than 50 kinds of MTs genes were found in different plants (Rauser 1999). MTs play an important role in chelating with heavy metals and in regulating Zn and Cd homeostasis in plant cells (Palmgren et al. 2008).
(2) PCs
   PCs were first extracted from the Cd stressed *Rauvolfia serpentine* cells by Grill et al. (1985). Researches show that PCs are a kind of hydrosulfuryl chelated polypeptide, consisting of homocysteine, glutamate, and glycine, known to be required for basic tolerance of metals in all plants. Due to high contents of hydrosulfuryl, PCs have a high metal affinity, and can be chelated with many heavy metal ions. The biosynthesis of PCs can be induced by many metals, including Cd, Ni, Pb, and Zn (Kalpheck et al. 1995). After a few seconds of heavy metal processing, plant cells will induce the generation of PCs, and then the low molecular PCs in the cytoplasm will be transported to the vacuole where PCs-metals are formed and stored (Sun et al. 2005; Hangavel 2007).
(3) LCs
   In addition to MTs and PCs, the third important category of metal chelating agents in plant is the LCs, including organic acids (such as oxalic acid, malate acid, citric acid, and amino acids), NA, and inorganic anions (e.g. phosphate). They play an important role in the cumulative mechanism of intracellular Zn with the ability of improving the heavy metal tolerance. LCs has been demonstrated to reduce free Zn^{2+} concentration by forming chelating compounds or precipitation. Studies have shown that citric acid is related with the accumulation and resistance of Zn^{2+} (Sanger et al. 1998) and malate acid is a Zn^{2+} combiner in the cytoplasm (Godbold et al. 1984). NA is a ubiquitous compound in plants and has the capacity to bind Zn, Fe, and other metals (von Wiren et al. 1999; Schaaf et al. 2004). Zhao et al. showed that citric acid has a high affinity of Zn^{2+}, and it can form the Zn-chelating groups in *Arabidopsis halleri* (Zhao et al. 2000). In *Thlaspi caerulescens*, up to 70 % of root Zn may be associated with His (Histidine) and the remaining 30 % was associated with the cell wall (Callahan et al. 2006).

Vacuoles are assumed to be the major sites of metal sequestration in root cells (Martinoia et al. 2007). Once Zn has entered the cytoplasm of a root cell, especially in excessive concentration, it might be transported into vacuoles and sequestered there as free Zn ions or organic complex. This process is also regulated by numerous metal ion transport systems. Members of cation diffusion facilitator (CDF) family of proteins have been inferred to be the key proteins controlling this process and in contributing to Zn tolerance in plant. The CDF family members ZRC1 and COT1, found in the *Saccharomyces cerevisiae*, are related with Zn^{2+} compartmentation in the vacuole by transporting Zn^{2+} from the cytosol to the vacuole (Conklin et al. 2003). Maestri et al. (2010) revealed that the MTP1 genes from *Thlaspi caerulescens*, *Arabidopsis halleri,* and *Thlaspi goesingense* are involved in increasing Zn sequestration by promoting the influx of Zn in the vacuole. Recent evidences suggest that expression of MTP1 might also respond to Zn deficiency, leading to increasing Zn uptake and accumulation (Gustin et al. 2009). Another protein MTP3 in *Arabidopsis* was found to play an essential role in Zn^{2+} tolerance and compartmentation (Arrivault et al. 2006). Other members of CDFs family, e.g., the $Mg^{2+}/H+$ antiporter AtMHX and the orthologs of the *Arabidopsis thaliana* Zn-induced facilitator 1 (AtZIF1) protein, can transport Zn^{2+} and Zn^{2+}-complexes separately into the vacuole (Maestri et al. 2010).

3.2.3 Translocation of Zinc from Root-to-Shoot

Water and mineral nutrients are primarily transported through the xylem in plant. Within the xylem, Zn is present and transported predominantly as Zn^{2+} and a complex with organic acids or NA. It was suggested that the xylem loading be the committed step in root export of metal ions to the shoot, partially controlling the concentration and distribution of Zn in plants. A series of transmembrane activities exist in these procedures that are regulated by variety of metal transporters intracellular or on the plasma membrane.

Phenotypic analysis of *Arabidopsis thaliana* mutants which carry the disruptions of genes HMA2 and HMA4 that code for two HMA (HMA: Heavy Metal Transporting ATPase) Zn pumps in the root parenchyma provides the strong evidence to confirm that xylem loading is the key step for Zn translocation from root to shoot (White and Broadley 2011). These HMAs are supposed to transport Zn across the plasma membrane of root vascular cells into the xylem prepared for transport to the shoot (Palmgren et al. 2008). The results revealed that the hma2 and hma4 genes double mutant greatly increases Zn contents in roots and decreases Zn contents in shoots, indicating the fact that relative gene coded Zn pumps are required for Zn translocation upwards (Hussain et al. 2004). Increasing the expression of Zn pumps coding genes like HMA2 and HMA4 in the *Arabidopsis thaliana* could be a positive attempt to enhance the rate of the root-to-shoot Zn translocation in Zn phytoremediation plants (i.e., the translocation factor). YSL proteins are also contributing to load Zn-NA complexes into the xylem (Curie et al. 2009). The Zn sequestered in the root vacuole is thought to be released through another class of metal transporter, members of the natural resistance-associated macrophage proteins (NRAMPs) family, including orthologs of the AtNRAMP3 and AtNRAMP4 transporters of *Arabidopsis thaliana* (Roosens et al. 2008; Verbruggen et al. 2009).

3.2.4 Zinc Distribution and Storage in Aerial Parts of Plant

Because of an effective metal excretion system lacking in plants, excessive Zn is transported to and compartmented in certain inactive metabolic organs or subcellular areas to avoid an excessive cytoplasmic Zn which can result in direct toxicities to the plant. Compartmentation of metals such as Zn, Cd, and Ni in the aerial parts has been regarded as one of the most probable mechanisms of metal detoxification in hyperaccumulating plants (Broadley et al. 2007).

Micronutrients like Zn have to be unloaded from the xylem and be actively taken up by living cells surrounding the xylem in order to enter the mesophyll in leaf cells and other organs. From these cells, micronutrients move from cell to cell until they reach their final destination in plants for their normal physiological functions or, if in excessive levels, are stored in specific locations like cell wall or vacuoles. Xylem-unloading processes are thought to be the first step in controlled distribution and detoxification of metals in shoots, as well as in a possible redistribution of metals via the phloem afterward (Schmidke and Stephan 1995). The distribution of metal ions within the leaf after unloading them from the xylem is found to be via the apoplastic or symplastic passage (Karley et al. 2000). The uptake of Zn by specific cell types within shoots is also facilitated by certain metal transport proteins. Members of the ZIP family are thought to mediate Zn^{2+} influx to leaf cells and also involve in the Zn loading into the phloem while YSL proteins are implicated in the same physiological process of Zn-complex in shoots (White and Broadley 2011).

Generally, concentrations of Zn in plant cells are within the specific physiological ranges, while excessive Zn can be sequestered in the aerial part of Zn hyperaccumulating plants. Intriguingly, the forms and distribution pattern vary between plant species. It was reported that in *Athyrium yokoscense* 70–90 % of Zn was stored in the cell wall in the form of ionic compounds or directly combined with the cell wall materials (Nishizono et al. 1987). However, in *Arabidopsis halleri* and *Thlaspi caerulescens,* the major Zn storage compartment is mesophyll tissues and vacuoles (Kupper et al. 1999; 2000).

Metal chelation in leaves shares the similar chelating mechanisms in the roots. A large number of metal chelators like organic acid, glutathione, PCs, NA, and proteins are produced or transported into the specific sites where excessive Zn exists, forming metal chelating complexes to keep a relatively steady Zn homeostasis in plant. The detailed mechanisms have been discussed in Sect. 2.2.

3.3 Phytoremediation of Zinc-Contaminated Soils

3.3.1 Zinc Contamination in Soil

With thousands of years of mining and processing, anthropogenic emission of Zn has become a primary cause for environmental Zn contamination. Zn is the fourth most common metal in use (after iron, aluminum, and copper) with an annual production of about 12 million tons (Tolcin 2011). About 70 % of the world's Zn originates from mining, while the remaining 30 % is from recycling. Globally, the major Zn mining countries are China, Australia, and Peru. China contributes 29 % to the global Zn production (Tolcin 2011). Zn is released into the environment through fossil fuel combustion, mine waste, phosphate fertilizers, limestone, manure, sewage sludge, and particles from galvanized surfaces. Soil Zn concentrations of over 1,000 mg kg^{-1} DW have been found in certain agricultural fields near industrial sites, compared with a background concentration of 100 mg kg^{-1} DW in agricultural soils (Audet and Charest 2006). Excessive Zn of 500 mg kg^{-1} in soil interferes with the ability of plants to absorb other essential elements, such as iron and manganese. Zn levels of 2,000–180,000 mg kg^{-1} (or 18 %) have been recorded in some Zn-contaminated soil samples (Emsley 2001).

3.3.2 Phytoremediation

Soil heavy metal contamination can be remediated by different chemical, physical, and biological techniques. The application of physical and chemical remediation technologies at contaminated sites likely cause adverse impacts on the ecosystem and generally the cost is relatively high for remediating a large polluted area. Among the numerous currently available remediation techniques, phytoremediation receives more attention as a cost-competitive, environmental-friendly, esthetically pleasing approach for site remediation.

Phytoremediation is using plants to take up, accumulate, store, degrade, or render organic or inorganic contaminants in contaminated soil and water by taking advantage of the natural abilities of plants. In general, phytoextraction, phytostabilization, phytodegradation, phytostimulation, and phytovolatilization are the five main subsets of phytoremediation that have been identified (Fig. 3.1) among which phytoextraction and phytostabilization are the most commonly applied processes for metal remediation. When applied in contaminated sites, one restriction to be considered is that phytoextraction is potentially feasible only in soils with low or moderate levels of contamination. However, for the heavily contaminated sites, phytostabilization with tolerant plants may be a more suitable strategy by stabilizing the contaminated sites and reducing the risk of erosion and leaching of pollutants (McGrath and Tunney 2010). Continuous or natural phytoextraction and chemically enhanced phytoextraction are two approaches that

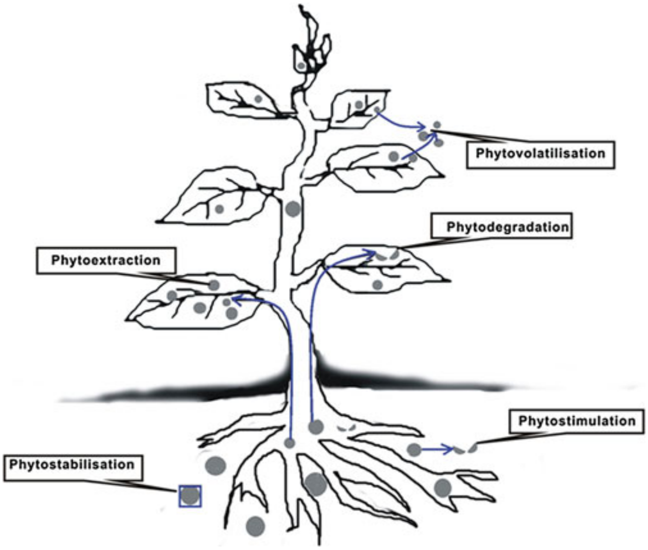

Fig. 3.1 Schematic diagram of main subsets of phytoremediation

have been proposed for phytoextraction of heavy metals (Lombi et al. 2001; Ghosh and Singh 2005)

Phytostabilization is a technique that can reduce the mobility and bioavailability of metal pollutants in the soil, aiming at decreasing the risks of pollutants to human health and the environment. Soil amendments, such as phosphate fertilizers, organic matter, Fe- and Mn-oxyhydroxides, and inorganic clay minerals can be applied to further enhance the reduction of metal bioavailability, preventing plants from absorbing or transporting in the surrounding environment (Berti and Cunningham 2000). Plants having extensive and abundant roots primarily accumulate pollutants in roots which are good candidate species for phytostabilization (Mendez et al. 2008).

Compared with phytostabilization, phytoextraction exploits the ability of plants to translocate a great fraction of metals taken up for harvesting biomass. Favorable plant properties for phytoextraction are generally fast growing, with high biomass production, an extended root system, and high translocation factor (TF, shoot-to-root metal concentration ratio), accumulation in harvestable tissues, and also easy agricultural management (Vamerali et al. 2010). Since it is more reliable to remove metals directly out of the contaminated sites than other physiochemical technologies, much interest is devoted to this technology and its improvement, while a better understanding of the physiological and molecular mechanisms in hyperaccumulators has inspired further improvement of the phytoremediation technology.

There are both advantages and disadvantages to the use of phytoremediation. Phytoremediation is less expensive than the traditional methods that clear the

Table 3.1 General physiological features observed in Zn hyperaccumulators

Different parts in plants	General features of Zn hyperaccumulators
In roots	Zn uptake rates are increased, generally reflected as an increased maxima velocity of absorption; Zn sequestration in the roots is decreased which may be induced by an enhanced root-to-shoot Zn transport; The rate of loading of Zn from the root into the xylem for root-to-shoot transport is strongly enhanced;
In shoots	Zn sequestration in the shoots, mostly inside the vacuoles of leaf cells, is highly effective; Cellular Zn uptake rates are enhanced, with a highly effective system for cell-to-cell Zn distribution.

contaminated sites by pumping, washing, or digging soil out of the contaminated site. This property makes phytoremediation even cheaper than traditional remediation methods. Since most plants used for phytoremediation are originally discovered on metal contaminated and uninhabitable sites, the wildlife there is able to flourish after being treated by those pioneers. But on the other hand, phytoremediation is restricted by the root depth of plants being used and can function only with low-to-moderate levels of contamination in the field. It may take many years to remediate the contaminated sites, so it is a time-consuming remediation method. Besides, low biomass and management of those applying plants are also the bottlenecks that restrict the implementation of this technology.

3.3.3 Zinc Hyperaccumulation

The first Zn hyperaccumulator species was reported in 1865, when Baumann et al. found that the Zn oxide contents reached up to 17 % in the ash of stem and leaf of *Thlaspi calaminare* (Sachs 1865). Since then more Zn hyperaccumulators have been identified. Baker et al. (1989) defined the plants that accumulate Zn more than 10,000 mg kg^{-1} DW (or 1 %, w/w) in the aerial parts as Zn hyperaccumulators. However, Broadley et al. (2007) recently suggested that 3,000 μg g^{-1} Zn concentrations (DW) in the aerial part might be more realistic. So far, among the reported metal hyperaccumulators, there are about 15–20 species for Zn, most of them in Brassicaceae (White and Broadley 2011). In addition to *Thlaspi calaminare*, *Arabidopsis halleri* is another most frequently studied Zn hyperaccumulator, which advances our understanding of Zn hyperaccumulation and hypertolerance at cellular and molecular levels (Kramer 2010). Yang et al. (2002) identified S*edum affredii Hance* as the potential Zn hyperaccumulator in China, with an average aboveground Zn concentration of 4,515 mg kg^{-1} DW in the field and the highest shoot Zn of 19,674 mg kg^{-1} DW in nutrient solution. The physiological processes of Zn uptake, transport to the xylem, and tolerance in shoot tissues are maximized in Zn hyperaccumulators. Certain features of Zn hyperaccumulators are summarized in Table 3.1.

Molecular mechanisms of Zn tolerance and hyperaccumulation are closely related to a set of constitutively highly expressed genes encoding metal transporters in the plasma membrane and enzyme-catalyzing compounds synthesis in physiological processes that facilitate Zn hyperaccumulating in those plants. So far, these Zn transporters have been found in several protein families, including ZIPs, YSLs, HMAs, ZIF1, MTPs, NRAMP (Yang et al. 2005; White and Broadley 2011). Compounds synthesized under certain enzymes catalysis (such as phytosiderophores and NA) are found to regulate Zn homeostasis by controlling the cell-to-cell mobility of Zn, excessive Zn sequestration in vacuoles, or other specific parts of plant cells. Taking one of the Zn hyperaccumulators, for example, upon comparison of *Thlaspi caerulescens* and *Arabidopsis thaliana*, ZIP4, ZIP10, and especially IRT3 (all from ZIP family proteins) were found to be much higher expressed in *Thlaspi caerulescens* roots than in *Arabidopsis thaliana* roots, even at different Zn exposures (van de Mortel et al. 2006). More molecular mechanisms and examples that are related to other metal transporters and enzymes regulation have been included in the part of "Physiological processes of Zn in plants".

3.3.4 Zinc Phytoremediation Strategies

Soil Zn contamination is a typical instance of metal pollution that can be cleaned up by phytoremediation. Many studies have been carried out both in the laboratory and in the field to develop Zn phytoremediation technology, but no successful full-scale application has been reported yet.

3.3.4.1 Natural Zinc Phytoremediation

Phytoremediation was first intended to utilize the natural properties of Zn-tolerant or hyperaccumulating plants to remove excessive Zn in the soil. Although promising, using this approach to remediate Zn contaminated sites is faced with many difficulties in the practical application. Lacking information on the agricultural management, slow growth rate, poor biomass of whole plants, and consuming a fairly long time and so on are only some of the barriers that block this application. Robinson et al. (1998) evaluated Zn uptake by *Thlaspi caerulescens* in pot trials and in wild populations at a mine waste site in France. It was estimated that the plant could remove 60 kg of Zn per ha per cropping, which was considered to be insufficient for Zn remediation. Brown et al. evaluated cadmium and Zn uptake by *Thlaspi caerulescens*, silene, and lettuce in 2-year-long field studies. The soils were contaminated by sewage sludge. A total of 18 growing-seasons will be needed to remediate a soil containing 400 mg Zn kg^{-1} (Brown et al. 1995).

Though most high yielding plant species have a relatively low tolerance for Zn, certain high biomass, and fast growing plant species may be the potential candidate species. Vamerali et al. (2010) reported that *Brassica*, *Zea mays,* and *Phaseolus vulgaris L.* could accumulate more than 1,000 mg Zn/kg DW. Sunflowers and maize have strong ability to take up Zn and other metals from the soils (Fellet et al. 2007; Tassi et al. 2008). Despite the superiority of certain field crops in high biomass compared to hyperaccumulator plant species, phytoremediation of Zn-polluted soils is still limited by the plant species under field conditions.

3.3.4.2 Improving Zinc Phytoremediation Efficiency

Plants used for phytoremediation are expected to have both high metal accumulation in shoots and high shoot biomass production (Vamerali et al. 2010). However, this ideal plant species has not been discovered. For this reason, promising physical, chemical, agricultural, and biotechnological approaches for enhancing the potential for Zn phytoremediation are explored either to improve the growth rate or biomass of phytoremediation plants. Phytohormones can be applied to promote root development and growth and further increase the whole plant growth or biomass production.

Chelating agents for enhancing phytoremediation (or phytorextraction) have been investigated to increase the heavy metal accumulation in plants having high biomass production and metal tolerance. Previous studies reported dramatic increases in plant Zn accumulation from soil in the presence of added synthetic chelates (Blaylock et al. 1997). The following order of extraction efficiency to Zn was achieved using biodegradable chelating agents to extract heavy metals from soil: NTA > EDDS > EDTA > MGDA > IDSA (Tandy et al. 2004). Unfortunately, without the appropriate management, using chelating agents to enhance phytoremediation could result in certain side effects, such as heavy metal leaching, reduced microbial diversity, and accumulation of refractory organic chemical chelating agents in the environment (Römkens et al. 2002).

Genetic engineering techniques that focus on improving growth and biomass production of known Zn hyperaccumulators are undergoing, though no successful case under field condition has been reported yet. Several targets are suggested for genetic engineering to improve Zn tolerance/accumulation in normal plant species, including overexpression of natural metal chelators (MTs, PCs, and LCs); regulating the metal transport systems on plasma membrane; alteration of the Zn metabolic pathways. Desbrosses-Fonrouge et al. (2005) identified the genes responsible for metal hyperaccumulating properties in model Zn/Cd hyperaccumulator *Thlaspi caerulescens*, which, if well characterized and properly expressed, could transform the high biomass producing species into metal tolerant and Zn accumulation. In an effort to correct for small sizes of hyperaccumulator plant, somatic hybrids have been generated between *Thlaspi caerulescens* and *Brassica napus*. The high biomass hybrid selected for Zn tolerance is capable of accumulating Zn to the level that would have been toxic to *B. napus* (Brewer et al. 1999).

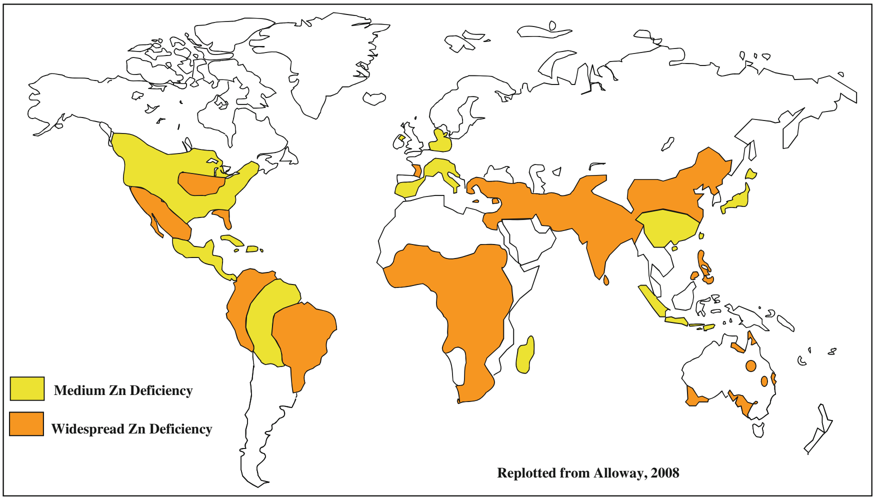

Fig. 3.2 Global distribution of Zn-deficiency affected regions (Replotted from Alloway 2008)

More studies will be needed to better understand the molecular and genetic mechanisms in hyperaccumulators of Zn and other metals, for setting the stage for the feasible and effective application of phytoremediation.

3.4 Zinc Biofortification for Human Nutrition

3.4.1 Zinc Deficiency in Human Body

In the early 1960s, Zn deficiency in the human body was first speculated with considerable supportive evidence (Prasad et al. 1963). Nowadays, nearly two billion people in the developing world are suffering from Zn deficiency. The global distribution of Zn-deficiency affected regions are shown in Fig. 3.2. Soil Zn deficiency is among the major global micronutrient deficiencies and has recently received more and more attention.

Zn deficiency is responsible for many health problems, including impairments of physical and mental growth, immune system, high risk of infections, DNA damage, and cancer development (Hotz and Brown 2004; Gibson 2006; Prasad 2007). The public health implications of Zn deficiency in the developing world are being for pursued for decades. It is now well established that Zn deficiency is responsible for diarrhea and pneumonia in children (Gibson et al. 2008; Walker and Black 2009). Retarded growth and dwarfishness are widely studied and considered to be the indicators of human Zn malnutrition especially in infants and young children. Besides, pregnant women are another group susceptible for Zn deficiency and a survey among 285 pregnant women in Haryana showed that 65 %

Table 3.2 RDAs for Zn

Age	Male (mg)	Female (mg)	Pregnancy (mg)	Lactation (mg)
0–6 months	2[a]	2[a]		
7–12 months	3	3		
1–3 years	3	3		
4–8 years	5	5		
9–13 years	8	8		
14–18 years	11	9	12	13
19 + years	11	8	11	12

[a] Adequate intake (AI)

of them suffer from Zn deficiency (Prasad 2010). Improving Zn malnutrition for the worldwide population, especially in developing countries has become an urgent task which calls for close international communication and cooperation between governments and research institutes.

Past efforts on agriculture production have primarily been focused on increasing crop yields; however, the accompanying decrease of mineral concentrations in grains was found as a new problem threatening the development of crop yields and even the food security. The ultimate goal of modern agriculture has been modified to produce nutritious foods sufficiently and sustainably (Zhao and McGrath 2009). The contents of nutrients in the edible parts of staple food crops, e.g. maize, rice, wheat, barley, contribute to the main mineral intake of people in the developing countries. Therefore, increasing concentrations of mineral elements, like Zn, Fe, and Se, in staple food crops is the most effective approach for public health to control malnutrition in Zn deficiency areas.

Recommended Dietary Allowances (RDAs) for Zn developed by the Food and Nutrition Board (FNB) at the Institute of Medicine of the US National Academies are displayed in Table 3.2 (Institute of Medicine, Food, and Nutrition Board 2001).

Although animal products, such as meat, fish, and poultry, contain more Zn than cereals, plant foods with low concentrations of Zn almost occupy the main foods in low developed countries. Zn concentrations in even the most favorable plant foods are inadequate to meet the requirements (Gibson et al. 1998). This problem is compounded by the limiting content of Zn and low Zn bioavailability in vegetarian diets.

3.4.2 Zinc Biofortification Strategies

To overcome Zn deficiency in humans, there are two main approaches: (i) changing dietary composition through dietary Zn supplements and (ii) biofortification on staple foods through increasing the Zn content of food grains by plant breeding. A wide public awareness and sustained funding from the government are required by the first approach, which makes it not so easy. Biofortification has no such difficulties and meantime dominates on the feasibility to be applied in both urban and rural areas. For example, for the millions of Zn-deficient people in South Asia, the major daily consumed staple crops, rice, and wheat, are chosen as the candidate for Zn biofortification to improve local malnutrition.

Biofortification is defined as a technology to improve the micronutrition contents in staple crops using traditional breeding practices and modern biotechnology. One way is genetic biofortification, including conventional breeding and genetic modification (GM), and the other is agronomic biofortification embodied as the application to micronutrients in fertilizers.

3.4.2.1 Breeding Strategies for Zinc Biofortification

With the abundant natural genetic variations and centuries of conventional breeding experiences, plant breeding strategy is widely accepted as a cost-effective and easily affordable solution among the stable food crops biofortification approaches. It is possible that breeding can increase Zn-tolerance in root and leaf crops and increase Zn mobility in the phloem of fruit, seed, and tuber crops. Currently, numbers of breeding programs are ongoing aimed at developing new cereal genotypes with high genetic ability to absorb Zn and also other micronutrients from soil and finally accumulate Zn in grain at desired levels. Plenty of organizations and research institutes are devoted to this challenging task, among which the HarvestPlus-Biofortification Challenge Program is one of the leading programs, aiming at improving stable food crops with Zn, Fe, and vitamin A, by using plant breeding strategy (Pfeiffer and McClafferty 2007).

Genetic variations in grain are essential for the development of new genotypes with high Zn concentrations. However, since cultivated crops contain narrow genetic variation for Zn accumulation, species with promising genetic resource for higher Zn concentration are needed. In a series of genetic variation collections of wild emmer wheat, quite a few wide wheat varieties are found with not only high concentrations of Zn in seeds but also with high tolerance to drought and Zn deficiency in soil (Peleg et al. 2008). A large genetic variation also exists in grain Zn concentration in different germplasms of other crops such as rice and maize, and related researches are undergoing in certain breeding programs (Graham et al. 1999).

In addition to traditional breeding, genetic breeding through GM also attracts the attention of researchers. The identification of certain genes that control physiological activities such as Zn uptake, translocation, distribution, and sequestration in plant (especially in some Zn-tolerant plants and Zn hyperaccumulators), together with the confirmation of numerous enzymes involved in the Zn homeostasis in plant, contribute to better understanding of the mechanism for Zn tolerance and accumulation in plant and also provide the theoretical basis for genetic breeding of grains with higher Zn concentration. A research about the transcription factors that regulate the adaptation to Zn deficiency in *Arabidopsis* *thaliana* speculates that the overexpression of bZIP19 and bZIP23 transcription factors could be used to increase Zn accumulation in edible portions of crops by inducing constitutive expression of a suite of Zn-deficiency responses (Assuncao et al. 2010). In *Arabidopsis thaliana*, when reducing the expression of AtHMA2, which is speculated to catalyze Zn^{2+} efflux across the membranes of root cells (Eren and Arguello 2004) or overexpressing the gene encoding AtHMA4, which is thought to load Zn into the xylem (Verret et al. 2004), and an increased leaf Zn concentration was observed. In another example, after the overexpression of HvNAS1 in tobacco, Zn concentrations in leaf and seed increased from 16 to 39 mg kg^{-1} DW and from 20 to 35 mg kg^{-1} DW, respectively (Takahashi et al. 2003). Several transgenic plants that have greater Zn concentrations in their edible tissues than conventional varieties have been created. A variety of cassava with roots Zn 40 mg kg^{-1} DW (Sayre et al. 2011), the brown rice with 56–95 mg Zn kg^{-1} DW (Vasconcelos et al. 2003; Johnson et al. 2011), and barley grain with 85 mg Zn kg^{-1} DW (Ramesh et al. 2004) have been reported.

However, in the implement of crops breeding, various environmental conditions can affect the effects of Zn biofortification during the long-term process over years. The adverse chemical and physical properties of cultivated soils reduce chemical solubility and availability of Zn in soils, resulting in inadequate amounts of Zn absorption from soils. Among the chemical factors, high soil pH is among the most critical factors reducing solubility and root absorption of Zn. Changing soil pH from 6 to 7 results in about a 30-fold decrease in soil Zn solubility and further significantly decreases plant Zn concentrations. Similar impairments in root absorption of Zn also take place in soils with low levels of soil moisture and organic matter. In Turkey, low annual rainfall (<300 mm), relatively high soil pH (7.5–8.1), and low soil organic matter (averaged 1.5 %) are responsible for the severe Zn deficiency in Central Anatolia (Cakmak et al. 1999). In many other cultivated soils around the world, such as in China, India, Iran, Pakistan, and Australia, similar Zn deficiency in soils has been also reported. Under such adverse environmental conditions, the newly cultivated plant with ability to accumulate high Zn in edible parts may not achieve the desired effect.

3.4.2.2 Agronomic Biofortification

Agronomic biofortification is generally known as the application of Zn fertilizers to soil and/or foliar to increase grain Zn concentrations. It is considered to be a flexible approach that can be used for all crop species and cultivars, and meantime an important complementation to the ongoing breeding programs of cereals with high Zn in grain. Compared to genetic biofortification, it is considered as a short-term solution without years of crossing and backcrossing activities. To gain high Zn in the grain by application of Zn fertilizers, two general conditions are required: first, keeping sufficient amount of available Zn in soil solution; second, maintaining adequate Zn transport to the seeds during the reproductive growth stage. The most widespread inorganic Zn fertilizer is zinc sulfate, along with zinc oxide

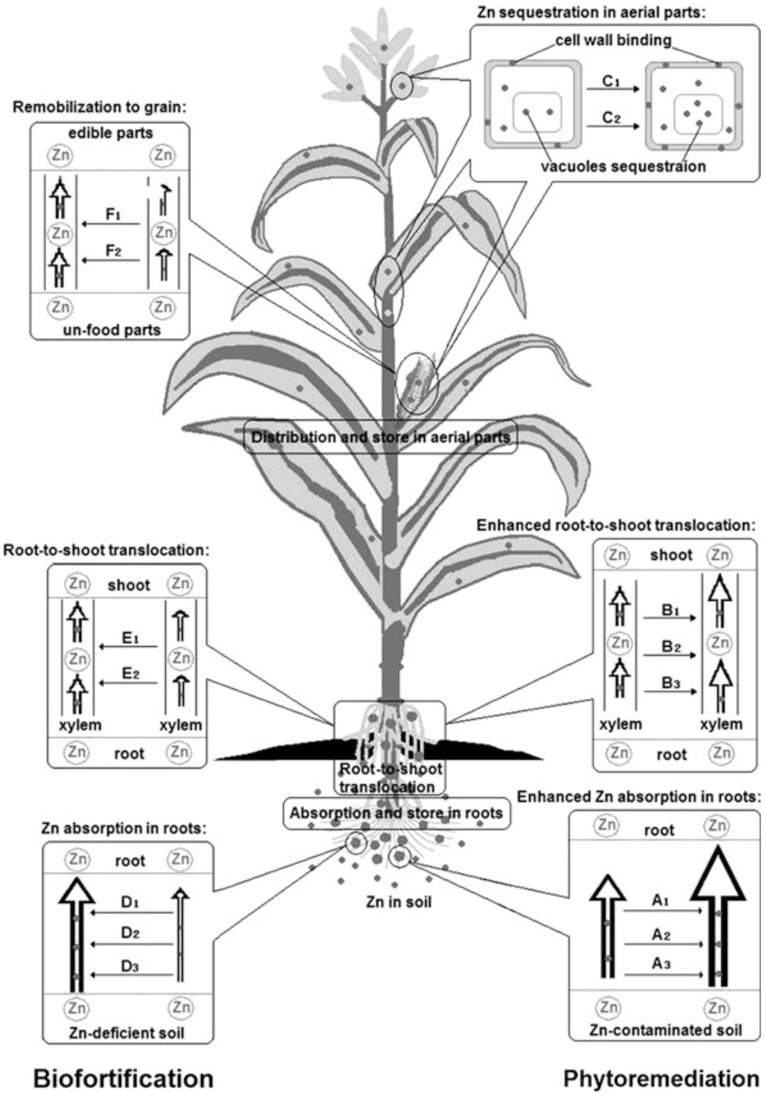

Fig. 3.3 Key processes and potential improvements in phytoremediation of Zn-contaminated soil and Zn-biofortification. A_1:Zn hyperaccumulators inherently absorb excessive Zn from soil to root; A_2: Increasing Zn bioavailability (1, decreasing soil pH; 2, enhancing microbial activities; 3, using synthetic Zn chelators); A_3: Highly expression Zn transporter genes on root cell membrane; B_1: Reduced Zn sequestration in roots; B_2: Enhanced Zn pumping into xylem (mediated by HMAs); B_3: Strong Zn demand signal in the shoots; C_1: Increased synthesis of Zn chelators in leaf cells; C_2: Excessive Zn sequestration in specific positions (vacuoles, cell wall); D_1: Application of Zn fertilizers; D_2: Increasing Zn bioavailability (similar to A_2); D_3: Traditional or genetic breeding to produce crops species with high Zn uptake ability; E_1: Identify traits responsible for efficient Zn translocation to shoots; E_2: Genetic transfer of potential governing factors of Zn xylem loading in Zn hyperaccumulators; F_1: Remobilization of Zn in non-food parts; F_2: loading more Zn into edible parts (like grains, fruits)

and synthetic Zn-chelates (White and Broadley 2009). Although the agronomic effectiveness of Zn fertilizers is higher with Zn-EDTA than the inorganic Zn fertilizer, its high cost and potential environmental adverse effects limit the use of Zn-EDTA in cereal farming. Safe and accurate application systems are required to process the fertilizers. When Zn fertilizers are applied in the soil, Zn phytoavailability and acquisition by roots should be considered for better Zn uptake. In addition to delivering phytoavailable Zn-fertilizer to the soil or foliage, certain agronomic strategies like reducing soil pH, adopting appropriate crop rotations, or introducing beneficial soil microorganisms contribute to high Zn phytoavailability (Rengel 1999; He and Nara 2007; White and Broadley 2009). When Zn-fertilizers are applied to foliage, it is particularly important to use soluble Zn compounds, making sure they can enter the leaf apoplast, and can be taken up by plant cells without having buildup on the foliar surface (Haslett et al. 2001; Cakmak 2008; Brown 2009).

There are a few convincing evidences on the role of Zn fertilizer application in improving grain Zn concentration. In Turkey, Zn biofortified wheat has been produced in field trials. Applying Zn fertilizers to wheat grown in fields in Central Anatolia not only improved productivity, but also increased grain Zn concentration (Yilmaz et al. 1997). Depending on the application method, Zn fertilizers can increase grain Zn concentrations up to 3- or 4-fold. The most effective method for increasing Zn in grain was to combine both soil and foliar application. When a high concentration of grain Zn is aimed in addition to a high grain yield, combined soil, and foliar application is recommended. Alternatively, using seeds with high Zn concentrations at sowing together with foliar application of Zn is also an effective way to improve both grain yield and grain Zn concentrations. New research programs are needed to develop or improve Zn application methods in terms of form, dose, and application time of Zn fertilizers.

3.5 Summary

Phytoremediation of Zn is an interdisciplinary technology that can benefit from many different approaches. The processes affecting metal availability, metal uptake, translocation, and chelation need further study. Since the inevitable involvement of engineering, biology, agronomic management even the use of chemical substances in the process of Zn phytoremediation, effective evaluation, and prevention strategies must be developed to avoid the possible negative impacts.

To increase Zn concentrations in edible crops by biofortification strategy, agronomic and genetic strategies should be integrated in the future. At the same time, a further identification of the mechanisms effecting general homeostatic regulation of tissue Zn concentrations within the plant and strategy of effective sequestration of Zn in non-vital compartments is required for the development of more feasible biofortification strategies. For this, a variety of lessons could be learnt from the studies of Zn phytoremediation especially those about Zn-tolerance and Zn-hyperaccumulation plants. The combination of breeding and fertilizer strategies is an excellent complementary approach to alleviate Zn-deficiency-related problems in human nutrition.

Both environmental Zn contamination and Zn malnutrition in humans are globally challenging problems that call for the concerted efforts of researchers in multiple fields, including plant biology, plant breeding, biotechnology, nutrition, and environmental sciences. Phytoremediation and biofortification of Zn as the promissing representative solution of each problem should have a mutual reference on mechanism and application. The simplified relation and distinction between phytoremediation of Zn contaiminated soil and biofortification of Zn for human nutrition are shown in Fig. 3.3.

Researches on Zn phytoextraction mainly take typical plants with the ability of Zn hyperaccumulating or Zn tolerance as the studying objects, but never stop the exploration of phytoremediating plants from general plants especially cereal plants with high biomass. For biofortification, cultivating cereal crops with satisfactory bioconcentration of Zn in the edible parts should take advantage of the existing positive research results of phytoremediation on the aspects of enhancing trace element bioavailability in the rhizosphere, translocation from roots to shoots, and further toward grain tissues. In both cases, significant progress can only be made through a better understanding of the underlying mechanisms of Zn acquisition, transport, and homeostasis in plants.

References

Alloway BJ (2008) Zinc in soils and crop nutrition. IZA Publications. International Zinc Association, Brussels, pp 1–116

Arrivault S, Senger T, Krämer U (2006) The Arabidopsis metal tolerance protein AtMTP 3 maintains metal homeostasis by mediating Zn exclusion from the root under Fe deficiency and zinc over supply. Plant J 46:861–879

Assuncao AGL, Herrero E, Lin YF et al (2010) *Arabidopsis thaliana* transcription factors bZIP19 and bZIP23 regulate the adaptation to zinc deficiency. Proc Natl Acad Sci U S A 107:10296–10301

Audet P, Charest C (2006) Effects of AM colonizationon “wild tobacco” plants grown in zinc-contaminated soil. Mycorrhiza 16:277–283

Auld DS (2001) Zinc coordination sphere in biochemical zinc sites. Biometals 14:271–313

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1(2):81–126

Baker AJM, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants. In: Shaw AJ (ed) Heavy metal tolerance in plants. CRC Press, Boca Raton, pp 155–178

Berti WR, Cunningham SD (2000) Phytostabilization of metals. In: Raskin I, Ensley BD (eds) Phytoremediation of toxic metals: using plants to clean up the environment. Wiley, New York, pp 71–88

Blaylock M, Salt DE, Dushenkov S et al (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci Technol 31:860–865

Bowen GC, Rovira AD (1991) The rhizosphere—the hidden half of the hidden half. In: Waisel Y, Eshel A, Kaffkafi U (eds) Plant roots—the hidden half. Marcel Dekker, New York, pp 641–669

Brady JE, Humiston GE, Heikkinen H (1983) General chemistry: principles and structure, 3rd ed. Wiley, p 671. ISBN 047186739X

Brewer EP, Saunders JA, Angle JS et al (1999) Somatic hybridization between the zinc accumulator *Thlaspi caerulescens* and *Brassica napus*. Theor Appl Genet 99:761–771

Broadley MR, White PJ, Hammond JP et al (2007) Zinc in plants. New philol 173:677–702

Brown P (2009) Development of a model system for testing foliar fertilizers, adjuvants and growth stimulants. In: Proceedings of the California department of food and agriculture fertilizer research and education program conference 2008. Visalia, CA, pp 17–23

Brown SL, Chaney RL, Angle JS et al (1995) Zinc and cadmiumuptake by hyperaccumulator *Thlaspi caerulescens* grown in nutrient solution. Soil Sci Soc Am J 59:125–133

Cakmak I (2000) Role of zinc in protecting plant cells from reactive oxygen species. New Phyl 146:185–205

Cakmak I (2008) Enrichment of cereal grains with zinc: agronomic or genetic biofortification? Plant Soil 302(1–2):1–17

Cakmak I, Kalayci M, Ekiz H et al (1999) Zinc deficiency as an actual problem in plant and human nutrition in Turkey: a NATO-science for stability project. Field Crops Res 60:175–188

Callahan DL, Baker AJM, Kolev SP et al (2006) Metal ion ligands in hyperaccumulating plants. J Biol Inorg Chem 11:2–12

Chen BD, Li XL, Tao HQ et al (2003) The role of arbuscular mycorrhizal in zinc uptake by red clover growing in a calcareous soil spiked with various quantities of zinc. Chemosphere 50:839–846

Clemens S, Palmgren MG, Krämer U (2002) A long way ahead: understanding and engineering plant metal accumulation. Trends Plant Sci 7(7):309–315

Conklin DS, Mcmaster JA, Culbertson MR et al (2003) COT 1, a gene involved in cobalt accumulation in *Saccharomyces cerevisiae*. Mol Cell Biol 12:3678–3688

Curie C, Cassin G, Couch D et al (2009) Metal movement within the plant: contribution of nicotianamine and yellow stripe1-like transporters. Ann Bot 103:1–11

Delorme TA, Gagliardi JV, Angel FS, Chaney RL (2001) Influence of the zinc hyperaccumulator *Thlaspi caerulescens J & C Presl* and the nonmetal accumlator *Trifolium Pratense L.* On soil microbial populations. Can J Microbiol 47:773–776

Desbrosses-Fonrouge AG, Voigt K, Schröder A et al (2005) *Arabidopsis thaliana* MTP l is a Zn transporter in the vacuolar membrane which mediates Zn detoxification and drives leaf Zn accumulation. Fed Eur Biochem Soc 579(19):4165–4174

Emsley J (2001) “Zinc”. Nature’s building blocks: an A–Z guide to the elements. Oxford University Press, Oxford, pp 499–505. ISBN 0-19-850340-7

Eren E, Arguello JM (2004) Arabidopsis HMA2, a divalent heavy metal-transporting PIB-typ ATPase, is involved in cytoplasmi Zn^{2+} homeostasis. Plant Physio 136:3712–3723

Farinati S, DalCorso G, Bona E et al (2009) Proteomic analysis of *Arabidopsis halleri* shoots in response to the heavy metals cadmium and zinc and rhizosphere microorganisms. Proteomics 9:4837–4850

Fellet G, Marchiol L, Perosa D, Zerbi G (2007) The application of phytoremediation technology in a soil contaminated by pyrite cinders. Ecol Eng 31:207–214

Ghosh M, Singh SP (2005) A review on phytoremediation of heavy metals and utilization of its byproducts. Appl Ecol Environ Res 3(1):1–18

Gibson RS (2006) Zinc: the missing link in combating micronutrient malnutrition in developing countries. Proc Nutr Soc 65:51–60

Gibson RS, Ferguson EL, Lehrfeld J (1998) Complementary foods for infant feeding in developing countries: their nutrient adequacy and improvement. Eur J Clin Nutr 52:764–770

Gibson RS, Hess SY, Hotz C et al (2008) Indication of zinc status at the population level, a review of the evidence. Brit J Nutr 99:14–23

Godbold DL, Horst WJ, Collins JC et al (1984) Accumulation of zinc and organic-acid in roots of zinc tolerant and non-tolerant ecotypes of deschampsia-caespitosa. J Plant Physiol 116(1):59–69

Graham R, Senadhira D, Bebe S et al (1999) Breeding for micronutrient density in edible portions of staple food crops: conventional approaches. Field Crops Res 60:57–80

Greenwood NN, Earnshaw A (1997) Chemistry of the elements, 2nd ed. Butterworth-Heinemann, Oxford. ISBN 0-7506-3365-4

Grill E, Winnacker EL, Zenk MH (1985) Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science 230:674–676

Grotz N, Fox T, Connolly E (1998) Identification of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency. Proc Natl Acad Sci U S A 95:7220–7224

Grotz N, Guerinot ML (2006) Molecular aspects of Cu, Fe and Zn homeostasis in plants. Biochimica et Biophysica Acta—Mol Cell Res 1763:595–608

Guerinot ML (2000) The ZIP family of metal transporters. Biochim Biophys Acta 1465:190–198

Gustin JL, Loureiro ME, Kim D et al (2009) MTP1-dependent Zn sequestration into shoot vacuoles suggests dual roles in Zn tolerance and accumulation in Zn-hyperaccumulating plants. Plant J 57:1116–1127

Hambidge KM, Krebs NF (2007) Zinc deficiency: a special challenge. J Nutr 137:1101–1105

Hangavel P (2007) Changes in phytochelatins and their biosynthetic intermediates in red spruce (Picearubens Sarg.) cell suspension cultures under cadmium and zinc stress. Plant Cell, Tiss Organ Cult 88:201–216

Haslett BS, Reid RJ, Rengel Z et al (2001) Zinc mobility in wheat: uptake and distribution of zinc applied to leaves or roots. Ann Bot 87(3):379–386

He XH, Nara K (2007) Element biofortification: can mycorrhizas potentially offer a more effective and sustainable pathway to curb human malnutrition? Trends Plant Sci 12(8):331–333

Holleman AF, Wiberg E, Wiberg N (1985) “Zink” (in German). Lehrbuch der Anorganischen Chemie (91–100 ed.). Walter de Gruyter, Berlin, pp 1034–1041. ISBN 3-11-007511-3

Hotz C, Brown KH (2004) Assessment of the risk of zinc deficiency in populations and options for its control. Food Nutr Bull 25:94–204

Hussain D, Haydon MJ, Wang Y et al (2004) P-type ATPase heavy metal transporters with roles in essential zinc homeostasis in *Arabidopsis*. Plant Cell 16:1327–1339

Institute of Medicine, Food and Nutrition Board (2001) Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc

Ishimaru Y, Suzuki M, Kobayashi T et al (2005) OsZIP4, a novel zinc-regulated zinc transporter in rice. J Exp Bot 56(422):3207–3214

Johnson AAT, Kyriacou B, Callahan DL et al (2011) Constitutive overexpression of the OsNAS gene family reveals single-gene strategies for effective iron- and zinc-biofortification of rice endosperm. PLoS One 6(9):e24476

Kalpheck S, Schlunz S, Bergnann L (1995) Synthesis of phytochelatins and homo phytochelatins in *Pisumsativum L.* Plant Physiol 107:515–521

Kapulnik Y (1996) Plant growth promotion by rhizosphere bacteria. In: Waisel Y, Eshel A, Kaffkafi U (eds) Plant roots—the hidden half. pp 769–781. Marcel Dekker, New York

Karley AJ et al (2000) Where do all the ions go? The cellular basis of differential ion accumulation in leaf cells. Trends Plant Sci 5:465–470

Kramer U (2010) Metal hyperaccumulation in plants. Ann Rev Plant Biol 61:517–534

Kupper H, Zhao FJ, McGrath SP et al (1999) Cellular compartmentation of zinc in leaves of the hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 119:305–311

Kupper H, Lombi E, Zhao FJ et al (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212:75–84

Lane BG, Kajioka R, Kennedy TD (1987) The wheat germ Ec protein is a zinc-containing metallothionein. Biochem Cell Biol 65(11):1001–1005

Lombi E, Zhao FJ, Dunham SJ et al (2001) Phytoremediation of heavy metal-contaminated soils: natural hyperaccumulation versus chemically enhanced phytoextraction. J Environ Qual 30(6):1919–1926

Macdiarmidc W, Gaither LA, Eide DJ (2000) Zinc transporters that regulate vacuolar zinc storage in *Sacchromyces cerevisiae*. EMBO J 19:2854–2855

Maestri E, Marmiroli M, Visioli G, Marmiroli N (2010) Metal tolerance and hyperaccumulation: costs and trade-offs between traits and environment. Environ Exp Bot 68:1–13

Maret W (2005) Zinc coordination environments in proteins determine zinc functions. J Trace Elem Med Biol 19:7–12

Martinoia E et al (2007) Vacuolar transporters and their essential role in plant metabolism. J Exp Bot 58:83–102

McGrath D, Tunney H (2010) Accumulation of cadmium, fluorine, magnesium, and zinc in soil after application of phosphate fertilizer for 31 years in a grazing trial. J Plant Nutr Soil Sci 173(4):548–553

Mendez MO, Maier RM (2008) Phytostabilization of mine tailings in arid and semiarid environments—an emerging remediation technology. Environ Health Perspect 116:278–283

Moffett BF, Nicholson FA, Uwakwe NC et al (2003) Zinc contamination decreases the bacterial diversity of agricultural soil. FEMS Microb Ecol 43(1):13–19

Moreau S, Thomson RM, Kaiser BN et al (2002) GmZIP1 encodes a symbiosis-specific zinc transporter in soybean. J Biol Chem 277(7):4738–4746

Nathalieal M, Hassinen NH et al (2001) Enhanced copper tolerance in *Silene vulgaris* (Moench) garcke populations from copper mines is associated with increased transcript levels of a b-type metallothionein gene. Plant Physiol 126:1519–1526

Nishizono H, Ichikawa H, Suziki S et al (1987) The role of the root cell wall in the heavy metal tolerance of *Athyriurn yokoseeme*. Plant Soil 101:15–20

Palmgren MG, Clemens S, Williams LE (2008) Zinc biofortification of cereals: problems and solutions. Trends Plant Sci 13:464–473

Peleg Z, Saranga Y, Yazici A et al (2008) Grain zinc, iron and protein concentrations and zinc-efficiency in wild emmer wheat under contrasting irrigation regimes. Plant Soil 306(1–2):57–67

Pfeiffer WH, McClafferty B (2007) HarvestPlus: breeding crops for better nutrition. Crop Sci 47:S88–S105

Pilon-Smits E (2005) Phytoremediation. Ann Rev Plant Biol 56:15–39

Prasad AS (2007) Zinc: mechanisms of host defense. J Nutr 137:1345–1349

Prasad AS (2008) Zinc in human health: effect of zinc on immune Cells. Mol Med 14(5–6):353–357

Prasad R (2010) Zinc biofortification of food grains in relation to food security and alleviation of zinc malnutrition. Curr Sci 98(10):1300–1304

Prasad AS, Miale A, Farid Z et al (1963) Zinc metabolism in patients with the syndrome of iron deficiency anemia, hepatosplenomegaly, dwarfism and hypogonadism. J Lab Clin Med 61:537–549

Ramesh SA, Choimes S, Schachtman DP (2004) Overexpression of an *Arabidopsis* zinc transporter in *Hordeum vulgare* increases short-term zinc uptake after zinc deprivation and seed zinc content. Plant Mol Biol 54:373–385

Rauser WE (1999) Structure and function of metal chelators produced by plants. Cell Biochem Biophys 31:19–48

Rengel Z (1999) Zinc deficiency in wheat genotypes grown in conventional and chelator-buffered nutrient solutions. Plant Sci 143(2):221–230

Robinson BH, Meblanc L, Petit D et al (1998) The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant Soil 203:47–56

Roosens NHCJ, Willems G, Saumitou-Laprade P (2008) Using *Arabidopsis* to explore zinc tolerance and hyperaccumulation. Trends Plant Sci 13:208–215

Römkens P, Bouwman L, Japenga J et al (2002) Potentials and drawbacks of chelate-enhanced phytoremediation of soils. Environ Pollut 116(1):109–121

Sachs J (1865) Handbuch der experimental-physiologie der Pflanzen. In: Hofmeister W (ed) Handbuch der physiologischen botanik. Engelmann, Leipzig

Saier MHJ (1999) A functional phylogenetic classification system for transmembrane solute ransporters. Microbiol Mol Biol Rev 64:354–411

Sanger S, Kneer R, Wanne RG et al (1998) Hyperaccumulation, complexation and distribution of nickle in *Sebertia acuminate*. Phytochemistry 47:339–347

Sayre R, Beeching JR, Cahoon EB et al (2011) The bio-cassava plus program: biofortification of cassava for Sub-Saharan Africa. Annu Rev Plant Biol 62:251–272

Schmidke I, Stephan UW (1995) Transport of metal micronutrients in the phloem of castor bean (*Ricinus communis*) seedlings. Physiol Plant 95:147–153

Schaaf G, Ludewig U, Erenoglu BE et al (2004) ZmYS1 functions as a proton-coupled symporter for phytosiderophore- and nicotianamine-chelated metals. J Biol Chem 279:9091–9096

Schaaf G, Schikora A, Häberle J et al (2005) A putative function for the *Arabidopsis* Fe-phytosiderophore transporter homolog AtYSL2 in Fe and Zn homeostasis. Plant Cell Physiol 46:762–774

Sugarman B (1983) Zinc and infection. Review of infectious diseases 5(1):137–147

Sun Q, Wang XR, Ding SM et al (2005) Effects of interactions between cadmium and zinc on phytochelat in and glutathione production in wheat (*Triticumaestivum L.*). Environ Toxicol 20:195–201

Takahashi M, Terada Y, Nakai I et al (2003) Role of nicotianamine in the intracellular delivery of metals and plant reproductive development. Plant Cell 15:1263–1280

Tandy S, Bossart K, Mueller R et al (2004) Extraction of heavy metals from soils using biodegradable chelating agents. Environ Sci Technol 38:937–944

Tassi E, Pouget J, Petruzzelli G et al (2008) The effects of exogenous plant growth regulators in the phytoextraction of heavy metals. Chemosphere 71:66–73

Tolcin AC (2011) "Mineral commodity summaries 2009: Zinc". United States Geological Survey. Retrieved 2011-06-06

Vamerali T, Bandiera M, Mosca G (2010) Field crops for phytoremediation of metal-contaminated land: a review. Environ Chem Lett 8:1–17

Van de Mortel JE, Almar Villanueva L, Schat H et al (2006) Large expression differences in genes for iron and zinc homeostasis, stress response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the related metal hyperaccumulator *Thlaspi caerulescens*. Plant Physiol 142:1127–1147

Vasconcelos M, Datta K, Oliva N et al (2003) Enhanced iron and zinc accumulation in transgenic rice with the ferritin gene. Plant Sci 164:371–378

Verbruggen N, Hermans C, Schat H (2009) Molecularmechanisms of metal hyperaccumulation in plants. New Phytol 181:759–776

Verret F, Gravot A, Auroy P et al (2004) Overexpression of AtHMA4 enhances root-to-shoot translocation of zinc and cadmium and plant metal tolerance. FEBS Lett 576:306–312

von Wiren N, Klair S, Bansal S et al (1999) Nicotianamine chelates both Fe-III and Fe-II. Implications for metal transport in plants. Plant Physiol 119:1107–1114

Walker CLF, Black RF (2009) Global and regional child mortality and burden of disease attributable to zinc deficiency. Eur J Clin Nutr 63:591–597

White PJ, Broadley MR (2009) Biofortification of crops with seven mineral elements often lacking in human diets—iron, zinc, copper, calcium, magnesium, selenium and iodine. New Phytol 182(1):49–84

White PJ, Broadley MR (2011) Physiological limits to zinc biofortification of edible crops. Front Plant Sci 2:1–11

Whiting SN, DeSouza MP, Terry N (2001) Rhizosphere bacteria mobilize Zn for hyperaccumulation by *Thlaspi Caerulescens*. Environ Sci Technol 35:3144–3150

White PJ, Whiting SN, Baker AJM et al (2002) Does zinc move apoplastically to the xylem in roots of *Thlaspi caerulescens*? New Phytol 153:201–207

White PJ, Bradshaw JE, Dale MFB et al (2009) Relationships between yield and mineral concentrations in potato tubers. HortScience 44(1):6–11

Yang XE, Long XX, Ni WZ et al (2002) *Sedum alfredii H*: a new Zn hyperaccumulating plant first found in China. Chin Sci Bull 47:1634–1637

Yang XE, Feng Y, He ZL et al (2005) Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation. J Trace Elem Med Biol 18:339–353

Yilmaz A, Ekiz H, Torun B et al (1997) Effect of different zinc application methods on grain yield and zinc concentration in wheat cultivars grown on zinc-deficient calcareous soils. J Plant Nutr 20(4–5):461–471

Zhao H, Eide D (1996a) The yeast ZRT 1 gene encode the zinc transporter protein of a high-affinity uptake system induced by zinc limitation. Proc Natl Acad Sci 93:2454–2458

Zhao H, Eide D (1996b) The ZRT2 gene encode a low affinity zinc transporter in *Saccharomyces cerevisiae*. J Biol Chem 271:23203–23210

Zhao FJ, McGrath SP (2009) Biofortification and phytoremediation. Curr Opin Plant Biol 12:373–380

Zhao FJ, Lombi E, Breedon T et al (2000) Zinc hyperaccumulation and celluar distribution in *Arabidopsis halleri*. Plant Cell Environ 23:507–514