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Biofortification and phytoremediation

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Producing nutritious and safe foods sufficiently and sustainably is the ultimate goal of modern agriculture. Past efforts have focused on increasing crop yields, but enhancing the concentrations of mineral micronutrients has become an urgent task because about half of the world population suffers from the malnutrition of iron, zinc, and selenium. Biofortification of these trace elements can be achieved through fertilization, crop breeding or biotechnology. On the other hand, soils contaminated with metals or metalloids may be cleaned up by phytoextraction that combines hyperaccumulation with high biomass production. Progress has been made in identifying inter-species and intra-species variation in trace element accumulation, and mechanistic understanding of some aspects of trace element transport and homeostasis in plants, but much remains to be elucidated.

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Introduction

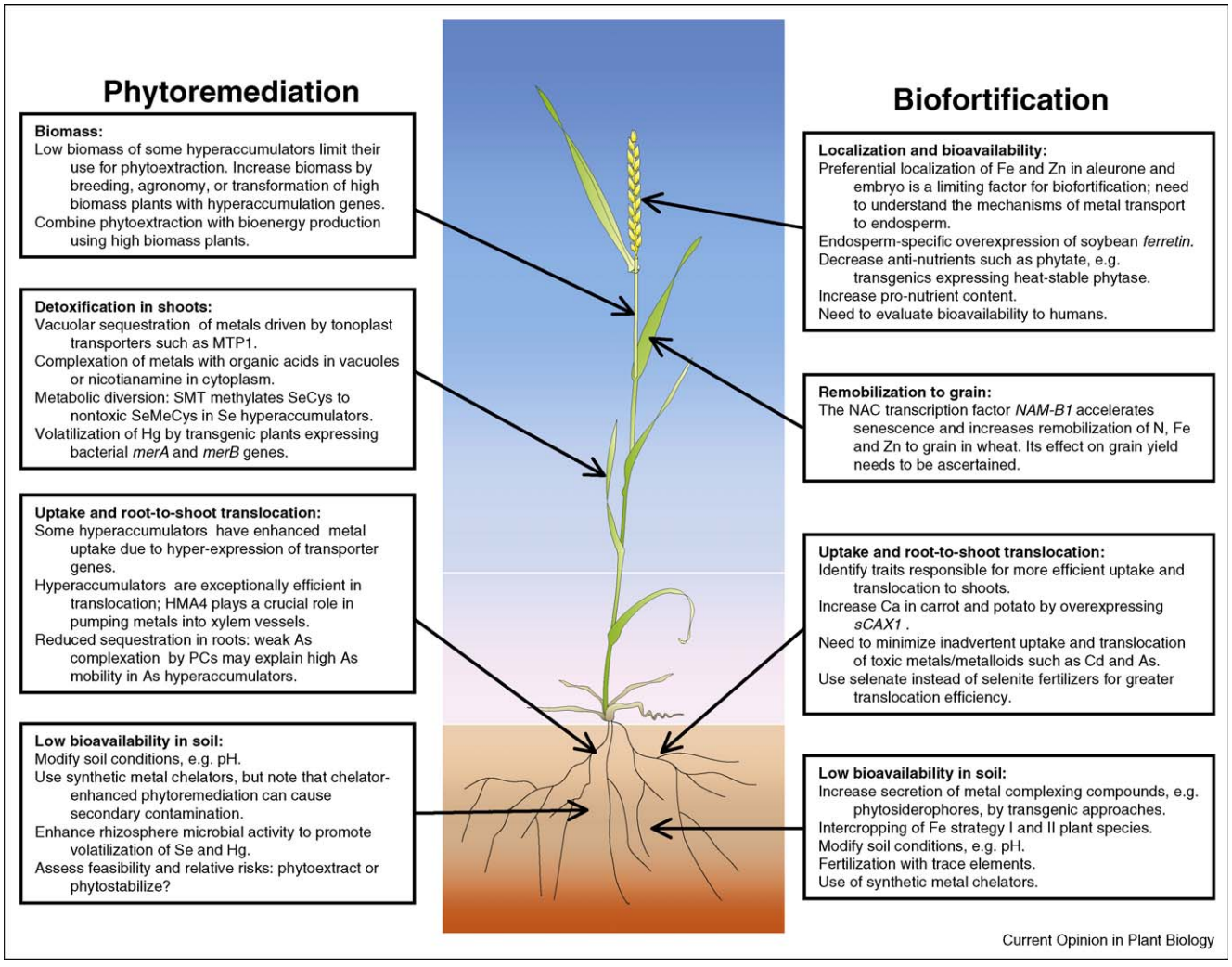
Biofortification of mineral micronutrients in food crops for the benefit of human nutrition and phytoremediation of metal/metalloid contaminated soils represent two potential biotechnological applications that arise from the research on mineral uptake, transport and metabolism in plants; these two very different goals are in fact the two sides of the same coin [1]. Understanding inter-species and intra-species variation in mineral uptake, distribution, metabolism and tolerance, and the molecular mechanisms responsible for the processes, is crucial for both applications. This brief review highlights recent progress in both topics, focusing on mineral trace elements that are either deficient for human requirement or toxic when present in excess. Key processes, bottlenecks, and potential solutions for both biofortification and phytoremediation are summarized in Figure 1.

Biofortification of mineral trace elements for human nutrition

Deficiencies of the mineral micronutrients iron (Fe), zinc (Zn), selenium (Se), and iodine (I) affect more than half of the world population [2]. Other minerals, such as calcium (Ca), magnesium (Mg), and copper (Cu), can also be deficient in the diets of some populations [3]. Past efforts have focused on increasing crop yields, but there is evidence that the increase in grain yield over the last four decades has been accompanied by the decrease in mineral concentrations in grain [4,5]. Biofortification, which aims to increase micronutrient concentrations in the edible parts of plants through breeding or the use of biotechnology, is considered to be a cost-effective way to alleviate micronutrient malnutrition in the rural populations in developing countries where the problem is most prevalent [6,7]. Biofortification may also include other approaches, such as the use of micronutrient fertilizers (agronomic biofortification) or enhancement of micronutrient bioavailability by manipulating the levels of pronutrient or antinutrient components in foods; the latter may represent a better strategy than attempts to increase mineral concentrations [8,9•].

For Zn and Fe, breeding for higher concentrations in grain is possible as there is sufficient genotypic variation in the germplasms of major cereal crops [3,10,11]. Wild emmer wheat shows a particularly large genetic variation in grain Zn concentration [12]. One of the key steps controlling grain concentrations of Zn and Fe is their remobilization from leaves and translocation to grain. Recent studies have shown that the *Gpc-B1* (Grain protein content-B1) locus from wild emmer wheat affects both grain protein content and the concentrations of Fe and Zn in grain [13]. This locus encodes a NAC transcription factor (NAM-B1) that accelerates senescence and increases remobilization of nutrients (N, Fe, and Zn) from leaves to developing grains [14••]. The wheat genome contains three *NAM* genes, but modern bread wheat varieties carry a nonfunctional *NAM-B1* allele, which causes delayed leaf senescence and lower levels of grain protein, Fe and Zn compared with wild emmer wheat. Introgression of the *Gpc-B1* locus into high yielding wheat cultivars is being carried out [12]. For Zn, agronomic biofortification may also be necessary on soils with low Zn availability, which represent nearly half of the cereal-grown areas in the world [12]. Because the majority of the Fe and Zn are localized in the aleurone and embryo of cereal seeds, which are removed by milling or polishing, it is important to ascertain if biofortification has actually increased the concentrations of these minerals in the endosperm, and has increased their bioavailability.

Figure 1



Key processes, bottlenecks, and potential solutions for phytoremediation of inorganic contaminants and biofortification of essential trace elements.

Transgenic approaches have also been actively pursued. An example is the endosperm-specific expression of the recombinant human lactoferrin (*rHLF*) gene in rice grain, resulting in the production of the HLF protein up to 0.5% of the grain weight and a twofold increase in grain Fe concentration [15]. HLF is a major protein found in human milk with a high-affinity for Fe binding, and is possibly involved in the regulation of Fe absorption [16]. The recombinant HLF protein produced in transgenic rice has the same bioavailability of Fe to humans as ferrous sulfate [16]. Another example is the endosperm-specific expression of the soybean *ferritin* gene in rice leading to twofold to threefold increase in the grain Fe concentration (e.g. [17,18]). Iron stored in plant ferritin is as bioavailable to humans as ferrous sulfate [9•,19]. Phytate, which accumulates in the aleurone and embryo cells of seeds, is the most important factor decreasing the bioavailability of Fe and Zn. Reducing phytate content in cereal and legume grain is possible, but tends to produce the undesirable consequence of lower yield and germination [9•]. An alternative is to express a phytase gene (*PhyA*) from microorganisms (e.g. *Aspergillus niger* or *Aspergillus fumigatus*) in the endosperm of cereal grain (e.g. [20]), or more usefully a heat-stable phytase so that the enzyme can better withstand high temperatures during cooking or processing and degrade phytate more efficiently [21]. Animal feeding experiments show a significant improvement in Zn bioavailability in the transgenic wheat containing a heat-stable phytase [9•]. Transgenic carrot and potato overexpressing the *Arabidopsis* H^+/Ca^{2+} transporter sCAX1, which has the N-terminal autoinhibitory domain truncated, were found to accumulate twofold to threefold more Ca in the edible parts of the plants than the wild-type [22,23]. Moreover, the increased Ca in the transgenic carrot is bioavailable to mice and humans [24•].

In the case of Se, agronomic biofortification offers the most effective solution [8]. This is because the main constraint on increasing the Se concentration in food crops lies in the low supply of Se from soil, which is often present in the form of selenite with relatively low bioavailability to plants, and when taken up by plants, tends to accumulate in roots [25]. Another reason is the insignificant variation in grain Se concentration among bread and durum wheat genotypes, making a strategy of breeding for biofortification difficult, although recent studies have shown that wild wheats including einkorn (*Tritcum monococcum* var. *monococcum*), emmer (*T. turgidum* var. *dicoccum*), spelt (*T. aestivum* var. *spelta*), and the wheat relative *Aegilops tauschii* contain higher levels of Se in grain than bread and durum wheats [26,27]. The Se concentration in cereal grains can be readily increased by the use of small amounts of Se fertilizers in the form of selenate, as has been practiced in Finland since the mid 1980s.

Phytoextraction of toxic metals and metalloids

Phytoremediation of land contaminated with inorganic and/or organic pollutants has attracted much attention and research over the last decade [28–30]. Among the various approaches comprising phytoremediation, phytoextraction of metals and metalloids is probably the most challenging task. Where soils are impacted by industrial or mining activities, the degree of pollution is usually severe, making phytoextraction unfeasible within a reasonable time frame because of the high quantity of the pollutants present in the soil. Simple mass-balance calculations show that phytoextraction is potentially feasible only in low or moderately contaminated soils. For more heavily contaminated soils, phytostabilization with tolerant plants may be used to stabilize the contaminated sites and reduce the risk of erosion and leaching of pollutants to water bodies. Hyperaccumulation of metals or metalloids is important for the phytoextraction strategy [31,32]. The last few years have seen a steady expansion in the list of hyperaccumulator species, which could be valuable plant resources for phytoremediation or for studies of mechanisms. However, a note of caution is warranted, as the hyperaccumulation ability of some reported 'hyperaccumulators' has yet to be confirmed in studies using field contaminated soils.

Chaney *et al.* [31] discussed situations where phytoextraction may be applicable, such as paddy soils contaminated with moderate levels of cadmium (Cd) giving rise to Cd concentrations in rice grain exceeding the safe limit. An Indica–Japonica hybrid cultivar of rice was found to be an effective Cd phytoextractor, removing 7–14% of soil Cd; this had the effect of decreasing subsequent Cd accumulation in soybean seeds in a pot study by 24–46% [33]. The efficient translocation of Cd from roots to shoots appears to be the main reason for the efficient Cd accumulation in this rice cultivar, although the underlying mechanisms remain unclear. A major QTL responsible for the root to shoot translocation of Cd has been identified in a F_2 population constructed from the parental lines of rice differing in Cd accumulation in shoots by 13-fold [34]. While the trait for high Cd accumulation in shoots is useful for phytoextraction, the opposite is true for the strategy of breeding crops low in Cd accumulation for the benefit of food safety [35]. Many of the previous studies on phytoextraction have focused on hyperaccumulators. Small-scale field trials have shown that an ecotype of the Zn/Cd hyperaccumulator *Thlaspi caerulescens* from southern France was able to phytoextract Cd efficiently in the seasons with good growth of biomass [36,37]. This ecotype possesses a high-affinity Cd uptake system that is not suppressed by Zn [38]. The Chinese brake fern *Pteris vittata* has a strong ability to hyperaccumulate arsenic (As) and shows promising potential for phytoextraction of As from contaminated soils under field conditions [39,40], but the plant thrives only in the humid tropic/subtropic climates. Phytoextraction using high biomass plants such as willow (*Salix* sp.) and poplar (*Populus* sp.) has also been proposed [41]. Some *Salix* species are good accumulators of Cd and Zn, and up to 20% of soil Cd could be removed by three croppings of *Salix* × *smithiana* in a lysimeter study [42]. A large proportion of the metals are stored in leaves, so plants either have to be harvested before leaf fall or the fallen leaves have to be collected. Such biomass plants may be grown on contaminated areas that are not suitable for food production, allowing gradual phytoextraction of metals while the biomass may be used to generate energy by pyrolysis. The method and temperature of pyrolysis need to be optimized to minimize volatilization losses of metals, among which Cd appears to be the most volatilizable [43].

Progress in understanding the mechanisms of hyperaccumulation

Hyperaccumulation of metals or metalloids is achieved through coordination of several processes, including enhanced metal uptake, efficient root-to-shoot translocation and effective detoxification in leaves. Transcriptomic analyses of the Zn/Cd hyperaccumulators *T. caerulescens* and *Arabidopsis halleri* show that a substantial number of genes involved in metal uptake and homeostasis are constitutively highly expressed compared with the non-hyperaccumulators *Thlaspi arvense* or *A. thaliana* [44–46], although it is possible that one or a very few genes control the expression of other downstream genes. It is now clear that the P_{1B}-type ATPases HMA4 and HMA2 play a crucial role in the root-to-shoot translocation of Zn and Cd, by pumping the metal ions from the pericycle cells to the xylem vessels [47,48••]. In the *Arabidopsis hma2, hma4* double mutant, Cd accumulation in shoots was almost completely abolished, indicating that the two HMAs are the major mechanism for root-to-shoot translocation of Cd [48••]. Knocking down HMA4 in *A. halleri* by RNAi

silencing resulted in a marked decrease in Zn and Cd accumulation in shoots, and a concurrent increase in the concentrations of these metals in roots [49••]. Hyperexpression of *HMA4* in *A. halleri* is attributable to a combination of modified *cis*-regulatory sequences and the expansion of the gene copy number. Manipulation of the HMA4 activity also impacts on the expression of the Zn-deficiency responsive genes in roots, which supports the idea that increased xylem loading drives an upregulation of transporter genes that are involved in metal influx. HMA4 has also been implicated in the tolerance of *A. halleri* roots to Zn and Cd [50,51]; this is confirmed by decreased root tolerance to these metals in the HMA4 RNAi lines of *A. halleri* [49••]. However, overexpression of *AhHMA4* in *A. thaliana* sensitized plant shoots to Zn and Cd as a result of enhanced root-to-shoot translocation. Detoxification in leaf cells requires other genes, possibly *MTP* members that encode tonoplast metal transporters [52]. Gustin *et al.* [53•] reported high levels of the MTP1 protein localized at the vacuolar membrane in shoot tissue of the Zn hyperaccumulator *Thlaspi goesingense*. When *TgMTP1* was expressed specifically in the shoots of the nonhyperaccumulator *A. thaliana*, it enhanced both Zn tolerance and accumulation in shoots; the latter was likely to be triggered by the activation of a systemic Zn-deficiency response as a result of increased vacuolar sequestration of Zn.

In the As hyperaccumulator *P. vittata*, As is translocated from roots to fronds extremely efficiently and mainly in the form of arsenite [54], presumably because little of the arsenite produced from the reduction of arsenate is complexed by phytochelatins (PCs) in roots and there is an efficient xylem loading system for arsenite [55]. The molecular mechanisms underlying the As hyperaccumulation phenotype in *Pteris* species remain largely unknown, which hampers the effort to transfer As hyperaccumulation genes to high biomass plants. A gene encoding an arsenate reductase (*PvACR2*) has been isolated from *P. vittata* [56]. Recombinant PvACR2 protein has *in vitro* arsenate reductase activity similar to its yeast homologue ScACR2, but the *in planta* function of PvACR2 remains to be investigated. A rice silicon efflux transporter Lsi2 has been shown to also mediate arsenite efflux from root cells towards xylem vessels [57••]. It remains to be investigated whether root-to-shoot translocation of arsenite in *Pteris* species involves Lsi2-like transporters. In nonhyperaccumulator plants, arsenite is complexed by PCs and sequestered in root vacuoles. Dhankher *et al.* [58] reported that *A. thaliana* with silenced arsenate reductase (AtACR2) accumulated 10–16-fold more As in shoots and retained less As in roots than the wild-type, possibly because knocking down this gene allows more arsenate to be loaded to the xylem via phosphate transporters. However, Bleeker *et al.* [59] observed the opposite phenotype with a decreased As accumulation in the shoots of the *AtACR2* knockout mutant. The *in planta* role of *AtACR2* remains unclear, as the bulk As speciation analysis showed little difference between the wild-type and the knockout mutants, both being dominated by trivalent arsenite [55].

Genetic engineering to enhance phytoextraction or phytovolatilization

Microbial or plant genes responsible for the transport, transformation, and tolerance of metals/metalloids can be overexpressed in high biomass plants to enhance their phytoremediation potential. Research by Meagher's group has demonstrated enhanced mercury (Hg) tolerance and phytovolatilization in transgenic plants expressing *Escherichia coli* mercuric ion reductase (*merA*) and organomercury lyase (*merB*) genes. This has been reviewed previously [29,60]. More recent progress includes the transformation of aquatic plants for Hg phytovolatilization in wetlands where pollution of Hg is more widespread [61], transformation in the fast-growing tree Eastern cottonwood (*Populus deltoides*) with both *merA* and *merB* genes [62], and the transformation of both genes into the chloroplast genome of tobacco [63,64]. Chloroplast transformation has several advantages over nuclear transformation, including the prevention of transgene escape via pollen to related species, high levels of foreign gene expression, and engineering multiple genes or pathways in a single transformation event [64]. The practical applications of Hg phytovolatilization are hampered by the perceived hazard of uncontained dispersion of a pollutant from soil to the atmosphere, even though the potential risk is considered to be minimal [62]. Additionally, the efficacy of this approach needs to be evaluated using field contaminated soils, which tend to have a low bioavailability of Hg.

Plants and the associated rhizosphere microbes may be used to take up and/or volatilize excessive build-up of Se in contaminated soil and irrigation drainage water [28]. Selenium, when present as selenate, is highly bioavailable to plant roots. Recent field trials have shown that transgenic *Brassica juncea* (Indian mustard) overexpressing genes involved in sulfur (S)/Se metabolism have enhanced Se accumulation and tolerance [65•,66•]. The transgenic plants overexpressing adenosine triphosphate sulfurylase (APS), which catalyzes sulfate/selenate activation before they can be reduced to sulfite/selenite [67], accumulated 4.3-fold more Se than the wild-type plants, and extracted approximately 4% of the extractable Se from a contaminated soil [65•]. Overexpression of APS and/or the APS reductase (APR) led to enhanced selenate reduction and assimilation [67,68]. However, there are conflicting reports regarding the effect of APS overexpression on Se accumulation and tolerance. Whilst Pilon-Smits *et al.* [67] reported an increased Se accumulation and tolerance in the APS transgenic Indian mustard, Sors *et al.* [68] observed the opposite phenotype in the APS transgenic *A. thaliana*. The major mechanism of Se toxicity in plants is the nonspecific incorporation of

selenocysteine (SeCys) and selenomethionine (SeMet) into proteins in place of Cys and Met, resulting in the alteration of protein structure [69]. One way to enhance Se tolerance is to direct the metabolic flow of SeCys away from protein synthesis by overexpressing SeCys lyase (SL), which decomposes SeCys to elemental Se and alanine [70,71]. When grown in a Se-contaminated soil under field conditions, the SL transgenic *B. juncea* accumulated approximately twofold more Se than the wild-type [66•]. Another way to engineer Se tolerance is to transfer the selenocysteine methyltransferase (SMT) gene from the Se hyperaccumulator *Astragalus bisulcatus*, which is also hypertolerant to Se, to nontolerant plants [72,73]. SMT catalyzes the methylation of SeCys to methylselenocysteine, which is a nonprotein amino acid nontoxic to plants. The SMT activity correlated strongly with the Se hyperaccumulation ability in eight species of *Astragalus* [68]. The SMT transgenic plants of *B. juncea* accumulated 60% more Se from a contaminated soil than the wild-type under field conditions [66•]. Interestingly, volatilization of Se was not enhanced in any of the above transgenic plants in field trials [65•,66•]. Transformation of *B. juncea* with both APS and SMT has also been attempted; the transformant was found to better accumulate selenate from the medium [74].

The soil bioavailability bottleneck

A common constraint for both biofortification and phytoextraction is the generally low bioavailability of trace elements in soil. Some plant species are able to secrete organic compounds to chelate metals in the rhizosphere soil, thus enhancing their solubility; the best-known example is the secretion of phytosiderophores (PS) by Graminaceous species in response to Fe deficiency. Note that this is a response to nutrient deficiency and, importantly, that Graminaceous species also possess the membrane transporters for Fe(III)–PS complexes [75]. Overexpression of the barley nicotianamine aminotransferase (NAAT, a key enzyme in PS biosynthesis) genes in rice enhanced the secretion of PS and Fe uptake from a calcareous soil low in Fe bioavailability [76]. Zuo and Zhang [77] showed that intercropping of dicotyledonous crops such as peanut or chickpea (strategy I plant species) with strategy II species such as maize or wheat enhanced Fe and Zn uptake and their concentrations in the seeds of dicotyledonous plants. It is possible that PS released by strategy II plants increases the concentrations of soluble Fe and Zn in the rhizosphere of strategy I plants. The ferric–PS can be reduced to ferrous ions by the ferric reductase of strategy I plants before being taken up through the strategy I pathway. The increased soluble Zn because of Zn–PS complexation would also increase the supply of free Zn^{2+} for the uptake by strategy I plants through dissociation of the complexes. In metal hyperaccumulators such as *T. caerulescens*, there is little evidence for the secretion of specific metal-chelating compounds [78]. Secretion of organic acids to the rhizosphere and subsequent complexation with metals can suppress metal uptake, as in the case of the Al tolerance mechanism employed by many plant species [79].

To overcome the bioavailability bottleneck, synthetic chelators such as EDTA have been used both to enhance the efficiency of micronutrient fertilizers applied to deficient soils and to promote phytoextraction of metals from contaminated soils [80,81]. For the latter, there have been many publications in recent years reporting various combinations of chelators, plant species, and metal contaminants. However, this approach is not environmentally friendly, because large amounts of chelators are needed to mobilize metals in the soil, and to force metal–chelator complexes to enter plants likely by damaging the plasma membranes of root cells and thus enhancing apoplastic uptake of the complexes. An unacceptable consequence of this approach is the persistence of metal–EDTA complexes in soil and potentially excessive leaching of the complexes to the subsoil and groundwater [82•] The uptake efficiency of metal–EDTA complexes by plants is also low compared with the amounts of metals mobilized in the soil. More recent research has focused on evaluating alternative chelators that are biodegradable and therefore less persistent in soil, with EDDS (ethylenediaminedisuccinic acid) being most studied [82•,83]. With the use of EDDS, leaching of metals is decreased, but not fully prevented, compared with EDTA [84], but the current price of EDDS is much higher than that of EDTA [31]. Chelator-assisted phytoextraction has not gained acceptance because of its relatively low efficiency, high risk of leaching and high cost of some chelators.

Conclusions

Micronutrient malnutrition in humans and environmental contamination with heavy metals or metalloids are both global and challenging problems that require concerted efforts from researchers in multiple disciplines including plant biology, plant breeding and biotechnology, nutrition, and environmental sciences. For biofortification, enhancing trace element bioavailability in the rhizosphere, translocation from roots to shoots, and redistribution towards grain tissues are the obvious targets [11,85]. For phytoextraction, combining the metal/metalloid hyperaccumulation traits with high biomass production is crucial, but for more contaminated soils phytostabilization may be the only feasible option. In both cases, significant progress can only be made through a better understanding of the underlying mechanisms of ion acquisition, transport, and homeostasis in plants. Furthermore, any potential technologies should be evaluated under real conditions. This means assessment of trace element bioavailability to humans in any biofortified products and potential downsides such as enhanced accumulation of toxic metals (e.g. Cd), which may share the same transporters as essential elements (e.g. Fe, Zn,

and Ca), and assessment of the efficacy and environmental risks of phytoextraction methods in contaminated soils under field conditions.

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