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Critical Review

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Hyperaccumulator plants from China: a synthesis of the current state of knowledge

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ABSTRACT

Hyperaccumulator plants are the material basis for phytoextraction research and for practical applications in decontaminating polluted soils and industrial wastes. China's high biodiversity and substantial mineral resources make it a global hotspot for hyperaccumulator plant species. Intensive screening efforts over the past 20 years by researchers working in China have led to the discovery of many different hyperaccumulators for a range of elements. In this review, we present the state of knowledge on all currently reported hyperaccumulator species from China, including *Cardamine violifolia* (selenium, Se), *Dicranopteris dichotoma* (rare earth elements, REEs), *Elsholtzia splendens* (copper, Cu), *Phytolacca americana* (manganese, Mn), *Pteris vittata* (arsenic, As), *Sedum alfredii* and *Sedum plumbizicola* (cadmium/zinc, Cd/Zn). This review covers aspects of the ecophysiology and molecular biology of tolerance and hyperaccumulation for each element. The major scientific advances resulting from the study of hyperaccumulator plants in China are summarized and synthesized.

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INTRODUCTION

The term 'hyperaccumulator' was first introduced into China in 1996,[1] exactly 20 years after it was used by Jaffré and his colleagues.[2] Since then, hyperaccumulators of metal(loid)s and rare earth elements (REEs) have attracted increasing academic and practical interests in China, as exemplified by the number of papers published by researchers working in China each year between 1997–2016 on this topic (Figure S1). In 2016, a total of 79 papers indexed in Science Citation Index (*i.e.* SCI-indexed) by researchers working in China focusing on hyperaccumulator plants were published, accounting for 41% of the total SCI-indexed papers on the subject by researchers worldwide (Figure S1). It was thus clear that significant efforts have been made in China to study hyperaccumulator plants, which is in part attributed to the potential for using hyperaccumulator plants for phytoremediation of metal-contaminated soils (a major pollution problem in China).[3] In fact, China has been engaged in developing and applying phytoremediation technologies at various scales, specifically large-scale phytoextraction of contaminants such as arsenic (As) and cadmium (Cd).[4,5] For examples, field trials have shown that: (*i*) after 7-month of growth in an As-contaminated soil, *Pteris vittata* was able to remove 7.8% of the total As in the soil (*i.e.* 0-20 cm layer of the soil);[6] (*ii*) after 13-month of growth in a Cd-contaminated soil, *Sedum plumbizincicola* could extract approximately 18% of the total Cd in the soil;[7] and (*iii*) after 35-month of growth in a Cd-contaminated soil, *Sedum alfredii* removed as much as 28% of the total Cd in the soil.[8] These results suggest that a substantial reduction in the concentrations of soil pollutants can be achieved by phytoextraction within a reasonable time frame, which is especially true for slightly or moderately contaminated soils.

DEFINING HYPERACCUMULATION STATUS AND POTENTIAL PITFALLS

Criteria for defining hyperaccumulation of trace elements have been proposed by Reeves,[9] Baker and Whiting,[10] and by Van der Ent *et al.*[11] In this review, we followed this terminology, *i.e.* a plant that has in excess of the following foliar concentrations (dry weight): 100 mg kg^{-1} for Cd, selenium (Se) and thallium (Tl); 1000 mg kg^{-1} for As, chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni) and REEs; 3000 mg kg^{-1} for zinc (Zn), and 10 000 mg kg^{-1} for manganese (Mn) in at least one specimen growing in its habitat while having the ability to complete its life cycle successfully. Although these values may appear arbitrary, they have proved useful for distinguishing abnormal from normal accumulation of metal(loid)s or REEs in plants.[12,13] This definition emphasized the fact that a plant should be hypertolerant to the element in question and survive without major toxicity symptoms to reproduce successfully. As such, work done solely on dosing plants in solution cultures or spiked soils where plants develop obvious toxicity due to breakdown of the metal regulatory network, but seemingly ‘hyperaccumulate’ are not confirmation of hyperaccumulation status.[14,15] Although a minimum concentration is needed to achieve uptake, the key characteristics of hyperaccumulator plants are their highly efficient uptake behavior and a non-linear dose response.[14,15] Provided dose levels are kept low (*e.g.* < 10 μM Co, Mn, Ni or Zn), hydroponic experiment is an important tool to identify genuine hyperaccumulators by confirming hypertolerance, hyperaccumulation in the shoot, high bioconcentration factor and high root-to-shoot metal translocation.[11,16] Indeed, the other two more strict criteria were proposed to screen out those genuine hyperaccumulators: (*i*) bioconcentration factor (*i.e.* the shoot-to-soil ratio of element concentration) is > 1; and (*ii*) translocation factor (*i.e.* the shoot-to-root ratio of element concentration) is > 1.[17] Other confounding issues can result from the contamination of foliar samples with extraneous soil or mineral particulates, an issue arising especially at mining and smelter sites from dust deposition.[11] Submerged (aquatic) plant species were not taken into account in this review, due to the risk of external contamination, which is exceedingly difficult to avoid.

OVERVIEW OF HYPERACCUMULATOR PLANT SPECIES FROM CHINA

The hyperaccumulator plant species reported by researchers working in China during 1997–2016 under both field conditions (*i.e.* natural habitats) and laboratory conditions (*i.e.* confirmatory experiments) are listed in Table 1. Whilst this overview is intended to be as comprehensive as possible, we do not seek to reach any definitive conclusions about the hyperaccumulation status of any given plant species. For example, *Solanum nigrum* has been reported as a Cd hyperaccumulator,[18] but was not included in Table 1 because Cd hyperaccumulation (≥ 100 mg kg^{-1}) has not been observed in above-ground tissue collected from natural habitats.

Under currently accepted definitions of recognized hyperaccumulator plant species, hyperaccumulators of As, Cd, Cu, Mn, REEs, Se and Zn have all been reported in China during the past 20 years (Table 1). The 29 hyperaccumulators listed in Table 1 were recorded in southern China, including Guangxi, Yunnan, Hunan, Zhejiang, Fujian, Guizhou and Sichuan Provinces (Table 1). Except for *Arabis paniculata*, *Picris divaricate*, *Sedum plumbizincicola* and *Viola baoshanensis*, these hyperaccumulator species are widely distributed in Asia or around the world (*e.g. P. vittata*). Caution must be applied when interpreting the data provided in Table S1 and those presented in the relevant literature (Figure S1). In many cases, only a few (< 10) samples of a given hyperaccumulator plant were analyzed. Strict washing methods were rarely employed for cleaning plant samples from the field (Table S1). In many instances, soils or nutrient solutions were artificially spiked with a given trace element in laboratory experiments (Table S1).

In this review, we focus on several well-studied hyperaccumulators when discussing aspects of ecophysiology and molecular biology of tolerance and accumulation in hyperaccumulators from China. Although there have been records of Pb hyperaccumulation in terrestrial plant species (see Table S1 for details), such instances will not be discussed here, given that the risk for external contamination and passive uptake of Pb are considered to be major confounding factors .[11,19]

ARSENIC HYPERACCUMULATORS

The occurrence of As hyperaccumulator plant species in China

The fern *P. vittata* (Figure 1) is the first As hyperaccumulator species identified in China.[20] Furthermore, all four As hyperaccumulators identified so far in China are in the pteridophyte genus *Pteris* belonging to the Pteridaceae family (Table 1).

Arsenic hyperaccumulation is a constitutive trait in *P. vittata*,[21] although there is variability in As accumulation between *P. vittata* populations (or genotypes).[22,23] Coincidently, the studies undertaken in China revealed considerable variations (up to 5.2-fold differences) in As accumulation between populations.[22,23] The experiments performed in China typically lasted for a longer time period than those undertaken outside China (84–160 days *vs* 30 days).[20,22-24] It has been observed that the shoot As concentration of a *P. vittata* population from Florida increased rapidly within the first 8 weeks after exposure to As and gradually approached a plateau in the following 12 weeks.[25] Further, non-metallicolous populations of *P. vittata* exhibited higher As accumulation but lower As tolerance than metallicolous accessions.[22,23]

Ecophysiology of As hyperaccumulation

Much like other hyperaccumulator plants, *P. vittata* has typical root exudates (such as oxalic acid) which enhance the solubility of As in its rhizosphere. More importantly, the root is able to exude phytic acid, an exudate that has not yet been observed in the rhizospheres of other hyperaccumulator plants.[26] Phytate exudation by *P. vittata* has been induced by As exposure and the addition of exogenous phytate into the rhizosphere of *P. vittata* led to a higher As concentration in the frond.[26] These observations suggest that *P. vittata* has developed a phytate-based strategy to solubilize As from its rhizosphere, thereby mediating efficient As uptake by the root and As hyperaccumulation in the frond (Figure 2).

Pteris vittata is able to take up both As(III) and As(V), but is more efficient in taking up the latter than the former (Figure 2).[27,28] As such, it was postulated that As(V) transporters have higher affinity for the substrate than As(III) transporters during rhizoid uptake.[27] There is evidence that non-metallicolous populations of *P. vittata* have lower *Km* (*i.e.* the Michaelis–Menten constant) but higher V_{max} (*i.e.* the maximum reaction velocity) for both As(III) and As(V) compared to metallicolous ones, indicating that the former generally have a greater number of As transporters per unit area of rhizoid plasma membrane than the latter.[29] This is consistent with the observation that non-metallicolous populations of *P. vittata* have a higher accumulation capacity for As than metallicolous populations.[22,23,29]

Arsenate can be taken up by the rhizoid of *P. vittata* via P transporters (Figure 2).[28] This contention is supported by the inhibitory effect of P addition on rhizoid uptake of As(V) by *P. vittata* in hydroponic culture.[30-32] In soil culture, the addition of either rock phosphates or P-fertilizers (such as NaH_2PO_4) could have positive, negative or zero effect on As uptake and translocation by *P. vittata*, which were attributed largely to the differences in physico-chemical properties of the soils tested and the doses of P added.[33-36]

The reduction of As(V) to As(III) is a key step in As detoxification pathways in rhizoids of *P. vittata* (Figure 2), although As(III) is considered to be more toxic in biological systems than As(V) and bioaccumulation of toxic As is harmful to not only the target species *per se* but also many other species in the food chain.[37] In agreement with this, As in the rhizoids of *P. vittata* is mainly coordinated with oxygen in the reduced state irrespective of As species added to the growth medium.[38] Furthermore, a more recent study demonstrated that As(V) reduction in *P. vittata* occurred mainly in the endodermis of fresh rhizoid.[32] The first evidence that arsenate reductase plays a major role in the reduction of arsenate in rhizoid of *P. vittata* was reported by Duan *et al.*,[39] which was confirmed subsequently by Liu and his colleagues.[40] There is increasing evidence that the rhizoid is the main location for As(V) reduction in *P. vittata*.[30,39,40] Furthermore, some endophytic bacteria isolated from *P. vittata* rhizoid are able to reduce As(V) to As(III) (Figure 2).[41] The reduction of As(V) may facilitate As sequestration in the vacuole, excretion of As out of the rhizoid cell and As loading into the xylem.[39]

About 60% of As in the rhizoid of *P. vittata* treated with As is distributed in cytoplasmic supernatant fraction (consisting mainly of the cell and vacuole sap) of the rhizoid cells,[42] supporting the notion that vacuolar compartmentalization is an important As detoxification pathway in the rhizoid. Furthermore, 85% of the total As in *P. vittata* callus exposed to As is present in its vacuole.[43]

Efflux of As(III) to the external environment is another As detoxification pathway in *P. vittata*.[44] Initially, weak efflux of As(III) from *P. vittata* rhizoid to the external environment was postulated, because the As(III) in As(V) solutions after being used for growing *P. vittata* accounted for a small portion of the total amount of As(V) added to the solutions.[30] However, increasing evidence suggests that the efflux of As(III) from *P. vittata* rhizoid could be strong and that bacterially-mediated As(III) oxidation in the growth medium make it difficult to accurately determine the amount of As(III) effluxed by the rhizoid.[45,46]

The majority (> 90%) of As in xylem sap of *P. vittata* is As(III) regardless of the As species added to the growth medium, suggesting a preferential loading of As(III) into xylem over As(V).[30] Additionally, there is evidence that both a high-affinity system and a low-affinity system are responsible for As(III) loading into xylem of *P. vittata*, with the former being dominant at low As(III) levels and the latter playing a leading role at high As(III) levels.[27] It is notable also that the presence of DNP (2,4-dinitrophenol) inhibited the As(III) xylem loading in *P. vittata* and the inhibitory effect decreased with increasing external As(III) concentrations, indicating a more important role of passive diffusion in the As(III) xylem loading at high As(III) concentrations.[27]

Several lines of evidence suggest that transpiration is involved in rhizoid-to-shoot translocation of As by *P. vittata* (Figure 2).[47] Firstly, the *P. vittata* population from a moister and warmer habitat (with an annual precipitation of 1736 mm and an annual temperature of 22°C) exhibited a higher transpiration efficiency and greater shoot As concentrations compared to that from a drier and cooler habitat (with an annual precipitation of 1426 mm and an annual temperature of 17°C), when they were grown in a culture solution containing 1 (one) mg As L^{-1}. Secondly, inhibition of transpiration by the addition of polyethylene glycol into the As-spiked growth media decreased shoot As concentration in both the two populations. Thirdly, the increment of transpiration by adding K into the As-spiked growth media enhanced shoot As concentration in the population with a lower transpiration efficiency.

The majority of As in the frond of *P. vittata* is coordinated with oxygen,[38] whilst vacuolar compartmentalization has been proposed as a major mechanism responsible for As storage and detoxification in the frond (Figure 2).[48] It was reported that 78% of the As accumulated in pinnae of *P. vittata* was distributed in cytoplasmic supernatant consisting mainly of cell and vacuole sap.[42] Further evidence was that 85% of the As taken up by callus of *P. vittata* was present in its vacuole.[43] Additionally, a quantitative proteomic analysis showed that three tonoplast proteins (one TDT transporter protein, a member of the TerC family, and a member of the ABC transporter G family) of *P. vittata* pinnae were upregulated by As(V), indicating the involvement of these proteins in vacuole compartmentalization of As (Figure 2).[49]

Molecular biology of As hyperaccumulation

Recently, a new *P. vittata* aquaporin gene (*PvTIP4;1*) was identified and shown to be responsible for As(III) uptake (Figure 2).[50] Heterologous expression in *Saccharomyces cerevisiae* suggested that PvTIP4;1 could act as an As(III) uptake channel, whilst transgenic *Arabidopsis* plants expressing *PvTIP4;1* produced less biomass but accumulated more As than the wild-type plants when they were exposed to As(III). Moreover, there is evidence that PvTIP4;1 was localized on the plasma membrane of *P. vittata* protoplasts and that *PvTIP4;1* transcripts were mainly expressed in the rhizoid of *P. vittata*. It was observed, however, that the expression of *PvTIP4;1* was not induced by As(III).[50] Such a phenomenon, in combination with the lack of effect of aquaporin inhibitors on As(III) uptake by *P. vittata* rhizoid,[51] indicates that the contribution of aquaporins in As(III) uptake by *P. vittata* needs further exploration. Despite this, *PvTIP4;1* is the only one known gene encoding a transporter that mediated As(III) uptake into the rhizoid of *P. vittata*.

Heterologous expression of *PvACR3* from *P. vittata* has shown that PvACR3 was localized on the plasma membrane of the transgenic *Arabidopsis* and likely mediated As(III) efflux from the cytoplasm of root and shoot cells as well as As(III) xylem loading of the transgenic plants.[52] These observations were inconsistent with those of Indriolo *et al*. who demonstrated for the first time that *PvACR3* encoded an As(III) transporter localizing to the vacuolar membrane in gametophytes of *P. vittata* and that this transporter was able to efflux As(III) into the vacuole for sequestration (Figure 2).[53]

Among the other four known genes from *P. vittata*, *PvPht1;3* identified by an American research team, is the only gene encoding an As(V) transporter.[54] The remaining three genes (*i.e. PvACR2*, *PvGRX5* and *Pv2.5-8*) from *P. vittata* were considered to encode As(V) reductases and reported by researchers outside China.[55-57]

CADMIUM HYPERACCUMULATORS

The occurrence of Cd hyperaccumulator plant species in China

In China, at least nine plant species belonging to seven families have been identified as Cd hyperaccumulators (Table 1), with *S. alfredii* (Figure 1) being the most studied species. A population of *S. alfredii* from Quzhou was first shown to hyperaccumulate Cd in 2004.[58] Another Cd hyperaccumulator belonging to the genus *Sedum* (*i.e. S. plumbizincicola*, Figure 1), found in Chunan and Quzhou in 2006, appears to be morphologically similar to *S. alfredii*.[59] However, nrDNA internal transcribed spacer sequence analysis has shown that *S. plumbizincicola* is indeed a taxonomic entity distinct from *S. alfredii*.[60]

Sedum alfredii occurs in both metalliferous and non-metalliferous soils and is hence a facultative hyperaccumulator.[61,62] Populations originating from metalliferous soils can accumulate one or two orders-of-magnitude more Cd compared to those originating from non-metalliferous soils.[61,62] In fact, Cd hyperaccumulation is not a constitutive trait in *S. alfredii* at the species level, similar to *Noccaea caerulescens* but not *Arabidopsis halleri*.[63,64]

Ecophysiology of Cd hyperaccumulation

Cadmium bioavailability in the rhizosphere of the hyperaccumulating population of *S. alfredii* was shown to be higher than that for the non-hyperaccumulating population.[65,66] The former was reported to be able to exude more dissolved organic matter into its rhizosphere than the non-hyperaccumulating population (Figure 3).[67] Increasing dissolved organic matter correlated with the decrease of pH in the rhizosphere (by 0.6–0.8 units), compared to the bulk soil.[66,68]

The K_m of ^{109}Cd influx into the root of the hyperaccumulating population of *S. alfredii* is similar to that of the non-hyperaccumulating population. However, the V_{max} is shown to be two-fold higher in the hyperaccumulating population.[69] These observations imply that the hyperaccumulating population has more Cd transporter proteins in its root membrane.

The responses of sulfur, cysteine and methionine metabolism to Cd exposure were much more pronounced in the root of the hyperaccumulating population of *S. alfredii* than in that of the non-hyperaccumulating population.[70] Despite this, only about 24% of the total Cd in the hyperaccumulating population root was complexed with glutathione.[71] These findings were consistent with the notion that a constitutive high level of glutathione biosynthesis could be important for alleviation of reactive oxygen species but not metal complexation in hyperaccumulator plants.[72]

Cadmium was found to be distributed preferentially in the root cylinder of the hyperaccumulating population but not in that of the non-hyperaccumulating population.[73] There was a 3–5-fold higher Cd concentration in xylem sap of the hyperaccumulating population than that of the non-hyperaccumulating population.[73] A greatly enhanced root-to-shoot translocation rate of ^{109}Cd was also observed in the hyperaccumulating population compared to that of the non-hyperaccumulating population,[69] suggesting that enhanced Cd xylem loading rate plays an important role in Cd hyperaccumulation by *S. alfredii*. [73]

Preferential Cd accumulation in the pith and cortex was observed in the stem of the hyperaccumulating population of *S. alfredii* (Figure 3), whereas Cd was restricted to the vascular bundles in the non-hyperaccumulating population.[74] In the leaf of the hyperaccumulating population, Cd was preferentially distributed in (photosynthetically active) parenchyma cells rather than in (photosynthetically inactive) epidermis.[71] This finding was remarkable, given that the foliar Cd hyperaccumulated by *A. halleri* and *N. caerulescens* was mainly concentrated in the trichomes and epidermal cells, respectively.[75-77] Such a seemingly abnormal phenomenon can be explained by the fact that the parenchyma cells of *S. alfredii* consist of large vacuolar cells which could serve as major storage sites for foliar Cd.[71] Moreover, the cellular distribution patterns of Cd in both the stem and leaf of the hyperaccumulating population were very similar to that of Ca but not Zn (Figure 3).[71,78] This phenomenon has not yet been observed in any other Cd hyperaccumulator, suggesting a possibly important role of Ca transporters in Cd hyperaccumulation by *S. alfredii* (Figure 3).[71] On the other hand, Cd in the leaf of the hyperaccumulating population of *S. alfredii* was found to be mainly associated with oxygen ligands (especially malic acid; Figure 3),[71] which is similar to the observations in *A. halleri* and *N. caerulescens*.[79,80]

Molecular biology of Cd hyperaccumulation

A P-1B type ATPase gene (*SaHMA3*) has been cloned and functionally characterized in *S. alfredii*.[81] *SaHMA3h* from the hyperaccumulating population encoded a Cd-specific tonoplast-localized transporter. It is similar to *TcHMA3* from *N. caerulescens* but not *AhHMA3* from *A. halleri*[82,83], because AhHMA3 is thought to be a Zn-specific tonoplast-localized transporter.[83] In contrast, SaHMA3n from the non-hyperaccumulating population was likely to transport not only Cd but also Zn. *SaHMA3* showed significantly higher constitutive expression in the hyperaccumulating population than in the non-hyperaccumulating population. These findings indicate that *SaHMA3h* plays an important role in facilitating Cd sequestration in the vacuole of *S. alfredii* (Figure 3).[81]

As a homology of *AtCAXs* encoding metal transporters localized in the tonoplast of *Arabidopsis thaliana*, *SaCAX2* in the shoot of the hyperaccumulating population of *S. alfredii* was transcriptionally upregulated when subjected to Cd.[84] However, the transcriptomes of *CAXs* in *A. halleri* and *N. caerulescens* seemed to be unaffected by Cd.[85,86]

The expression patterns of certain genes involved in S-assimilation are regulated by Cd in both the hyperaccumulating and non-hyperaccumulating populations of *S. alfredii*.[70] The transcript level of *NAS* encoding nicotianamine synthase (NAS) in the root of the hyperaccumulating population is 141-fold higher than that in the non-hyperaccumulating population, whereas a 0.3-fold difference was observed between the shoots.[70] These results suggest that nicotianamine plays an important role in Cd tolerance of the hyperaccumulating population of *S.*

alfredii.[70] In contrast, a recent study provided little evidence that nicotianamine was important for Cd tolerance in *A. halleri*.[87] Additionally, the transcript level of *SaMT2* (a type II metallothionein gene) in the Cd-hyperaccumulating population of *S. alfredii* was higher in the shoot than in the root, being induced by Cd treatments.[88] This finding is inconsistent with the observation that the expression of *MT2* in *N. caerulescens* was hardly affected by Cd exposure.[89] Taken together, these discrepancies indicate that the molecular mechanisms underlying Cd hyperaccumulation by *S. alfredii* differ in many aspects from those of *A. halleri* and *N. caerulescens*.

ZINC HYPERACCUMULATORS

The occurrence of Zn hyperaccumulator plant species in China

Sedum alfredii is the first hyperaccumulator of Zn identified in China.[90,91] The discovery of the Zn hyperaccumulation capacity of *S. alfredii* was about 3 years earlier than that of its Cd hyperaccumulation capacity.[58] Indeed, six of the seven Zn hyperaccumulators identified so far in China are also able to hyperaccumulate Cd (Tables 1 and S1).

There is a remarkable difference between the Zn-accumulating abilities of different *S. alfredii* populations. Even for metallicolous populations collected from mining areas with similar Zn levels in soils, the Quzhou population can accumulate > 10 000 mg kg^{-1} Zn in its shoot, whilst the Jinchuantang (Hunan Province) population accumulates < 2000 mg kg^{-1} Zn.[66] This variation suggests that Zn hyperaccumulation is not a constitutive trait in *S. alfredii*, in contrast to *A. halleri* and *N. caerulescens* which exhibit constitutive Zn hyperaccumulation.[63,64] Contrary to the Quzhou population, the non-metallicolous population of *S. alfredii* from Hangzhou (Zhejiang Province) is unable to accumulate > 1000 mg kg^{-1} Zn in its shoot, and the accumulated Zn is mostly allocated within its root.[92] Thus, the Hangzhou population has been widely adopted to make comparisons with hyperaccumulating populations to reveal the possible mechanisms underlying Zn hyperaccumulation and hypertolerance of this species.

Ecophysiology of Zn hyperaccumulation

After exposure to Zn, the root volume and diameter of the hyperaccumulating population of *S. alfredii* increased significantly (Figure 3), whereas the opposite was true for the non-hyperaccumulating population.[65,93,94] Furthermore, the Zn concentration in the shoot of the hyperaccumulating population was found to be positively correlated with the root length, surface area and volume.[93] In a soil with heterogeneous Zn distribution, the hyperaccumulating population allocated 90% of its root biomass into Zn-enriched areas.[95] This foraging adaptation might contribute to the efficient Zn uptake by the root.

The bioavailability of Zn in the rhizosphere of the hyperaccumulating population of *S. alfredii* was demonstrated to be greatly enhanced compared to that of the non-hyperaccumulating population, which was due largely to a decrease in rhizosphere pH (by 0.6–0.8 units) driven by more dissolved organic matter.[70] Rhizosphere bacteria of the hyperaccumulating population have been isolated and shown to be able to mobilize insoluble Zn by decreasing pH through production of short-chain organic acids.[96] Some isolated endophytes are also able to solubilize Zn directly from minerals such as $ZnCO_3$ and $Zn_3(PO_4)_2$, implying their positive roles in the root Zn activation.[96]

Compared to the non-hyperaccumulating population of *S. alfredii,* Zn influx in root was found to be 3-fold greater in the hyperaccumulating population,[97] which indicates an enhanced root Zn uptake system. Further analysis revealed a higher V_{max} for Zn in the root of the hyperaccumulating population.[93] About 2-fold more Zn was accumulated in the root cell vacuole of the non-hyperaccumulating population than in the hyperaccumulating population, whereas Zn efflux from the root cell vacuole was almost 2-fold slower in the non-hyperaccumulating population than in the hyperaccumulating population.[98] As such, more Zn was retained in the root of the non-hyperaccumulating population than in that of the hyperaccumulating population.[97]

Zinc is largely compartmentalized in root cell wall for both the hyperaccumulating and non-hyperaccumulating populations of *S. alfredii*.[99] However, in comparison to the non-hyperaccumulating population, the hyperaccumulating population bound Zn more loosely to its root cell wall, thereby Zn was more readily loaded into the xylem and then translocated to its shoot.[99]

At the initial stage of Zn exposure, significantly more Zn is allocated in the stem vascular bundle of the hyperaccumulating population of *S. alfredii* compared to that of the non-hyperaccumulating population, indicating a faster root-to-shoot Zn translocation in the hyperaccumulating population through the vascular bundle (Figure 3).[100,101] As the exposure time progressed, Zn accumulation in the vascular bundle tended to level off towards saturation, yet that in the stem epidermis of the hyperaccumulating population increased rapidly, leading to high concentrations of Zn in both the vascular bundle and the epidermis. Similarly, in the stem of *S. plumbizincicola*, Zn was mainly accumulated in the epidermis and in the vascular bundle and neighbouring parenchyma cell.[102]

In the leaf, Zn was also preferentially accumulated in the epidermis of both population types of *S. alfredii*, but to a much greater extent in the hyperaccumulating population.[100] Similarly, Zn was found to accumulate preferentially in the leaf epidermal cell of *S. plumbizincicola*.[103] The preferential distribution of foliar Zn in epidermal cell has also been reported for *A. halleri* and *N. caerulescens*.[75,104]

Concentration of glutathione in the leaf was found to increase with increasing Zn concentrations in growth media for both of the two population types of *S. alfredii*.[105,106] However, it was shown that the dominant chemical form of Zn in the leaf of the hyperaccumulating population was Zn malate (Figure 3).[107] This finding is similar to that for *A. halleri* but not *N. caerulescens* (where foliar Zn was present predominantly in the form of another carboxylic acid; Zn citrate).[108,109]

Molecular biology of Zn hyperaccumulation

Two members of *ZIP* (zinc-regulated transporter, iron-regulated transporter protein) gene family, *i.e. SaZIP2* and *SaZIP3*, were cloned from the hyperaccumulating population of *S. alfredii*.[110] Heterologous expression of the two genes showed that they were encoding transporters localized on plasma membrane and that the corresponding ZIPs could transport not only Zn but also Cd. The transcripts of *SaZIP2* and *SaZIP3* in the root and shoot of *S. alfredii* were regulated by Zn and Cd status in growth media, which is inconsistent with the finding that many ZIPs in *A. halleri* and *N. caerulescens* are expressed more or less independently of Zn status in growth media.[111,112] Nonetheless, these results provide evidence for the involvement of ZIPs in Zn and Cd hyperaccumulation by *S. alfredii* (Figure 3).[110]

A metal tolerance protein encoding gene (*MTP1*), which was homologous to *AtMTP1* encoding a Zn transporter localized on the tonoplast of *A. thaliana*, has been cloned from both the hyperaccumulating and non-hyperaccumulating populations of *S. alfredii*.[113] Heterologous expression of *MTP1* from *S. alfredii* was able to suppress Zn hypersensitivity in a *S. cerevisiae* mutant. The transcript of *MTP1* in the shoot of the hyperaccumulating population was upregulated by Zn, whereas its transcript in the root seemed to be affected only slightly by Zn. In contrast, an elevated transcript level of *MTP1* associated with Zn exposure was observed in the root but not in the shoot of the non-hyperaccumulating population.[113] These results suggest an important role of *MTP1* in Zn sequestration in vacuole of the shoot of the hyperaccumulating population (Figure 3), which is comparable to that in *A. halleri* and *N. caerulescens*.[112,114] Although *HMA4* has proven critical for

Zn hyperaccumulation in *A. halleri*,[115] a homology of *AhHMA4* from *S. alfredii* still remains to be characterized.

HYPERACCUMULATORS FOR OTHER ELEMENTS

Copper hyperaccumulators

Commelina communis growing in Cu mining wastelands located in Tongling, Anhui Province has been found to accumulate 731 (range 369–831) and 547 (429–587) mg kg^{-1} Cu in its stem and leaf, respectively.[116] Based on these field data, *C. communis* was considered initially as a Cu hyperaccumulator. Note, however, that a subsequent field survey showed that the average concentration of Cu in the shoot of a *C. communis* population from 'Tonglvshan' (Hubei Province) was 1224 mg kg^{-1}.[117] This population was then found to grow well in solutions containing up to 51 mg Cu L^{-1} and to be able to accumulate ~540 and 1300 mg kg^{-1} Cu in leaf and stem, respectively.[118] Copper hyperaccumulation has also been observed in another *C. communis* population growing in the Baoshan mine located in Tongling.[119] Low shoot Cu concentrations were recorded for *C. communis* populations from both contaminated and uncontaminated sites. For example, concentrations of Cu in the stem and leaf of a *C. communis* population growing in Tangshan Cu mine (Jiangsu Province) were only 40 and 28 mg kg^{-1}, respectively.[120]

Copper hyperaccumulation in *Elsholtzia splendens* (Figure 1) was originally observed in an old Cu mine located in Zhuji (Zhejiang Province).[121] In a subsequent study, the Zhuji population of *E. splendens* was found to accumulate ~90 mg kg^{-1} in its leaf after growing for 24 days in a nutrient solution containing 6.36 mg Cu L^{-1} (the highest Cu concentration tested that did not induce toxicity symptom or biomass reduction).[122] There have been few, if any, reports of Cu hyperaccumulation in other populations of *E. splendens*. In contrast, at least four populations growing in Cu mines located in Anhui, Hubei and Jiangsu Provinces were found to accumulate < 500 mg kg^{-1} Cu in their stems and leaves.[117,123]

Copper in *E. splendens* is shown to be bound predominantly by nitrogen/oxygen ligands rather than sulfur ligands.[124] Similarly, Cu in *Crassula helmsii* (a newly reported Cu hyperaccumulator outside China) was found to be bound almost exclusively to oxygen ligands.[125] Whereas antioxidative enzymes (*i.e.* CAT, SOD and POD) were reported to play a minor role in Cu tolerance of the hyperaccumulating population of *C. communis* from Tonglvshan,[126] a gene that is likely to encode calmodulin was considered to be involved in the Cu tolerance of this population.[127]

Manganese hyperaccumulators

The first Mn hyperaccumulator discovered in China is *P. americana* (synonym of *P. acinosa*, Figure 1),[128-130] which is the first known herbaceous Mn hyperaccumulator globally.[131] It can accumulate up to 14 477 mg kg^{-1} Mn in its leaf when growing naturally at a Mn mine located in Xiangtan, Hunan Province.[128-130] Most work has been confined to *P. americana*, although four other Mn hyperaccumulators have been reported in China (Tables 1 and S1).

There are several lines of evidence that transpiration plays an important role in root-to-shoot translocation of Mn in *P. americana* and distribution of Mn in the leaf.[132] Firstly, concentrations of Mn in a mature leaf of *P. americana* decreased by ~90% when its transpiration was inhibited by wrapping the leaf with a transparent plastic bag. Secondly, Mn concentrations were higher in mature leaves with larger leaf surface area (higher transpiration rate) than in young leaves. Thirdly, the concentration of Mn in the marginal part (the end of transpiration stream) of a leaf was greater than that in the midrib.

A cell-fractionation analysis of Mn distribution in the leaf of *P. americana* suggested that about 80% of the foliar Mn was found in the final supernatant fraction consisting mainly of vacuole and cytoplasm.[133] Using X-ray absorption spectroscopy, it was further shown that nearly 90% of the Mn in the final supernatant fraction was present in the form of Mn oxalate. This finding is inconsistent with the observation that malate or citrate is a major complexing ligand for Mn in other Mn hyperaccumulators from Queensland, Australia (*e.g. Gossia bidwillii* and *Virotia neurophylla*).[134] Additionally, *P. americana* differed from other Mn-hyperaccumulator plants in foliar Mn sequestration. The concentrations of Mn in the epidermal cell of *P. americana* were higher than those in mesophyll,[133] whilst the opposite was true for other Mn hyperaccumulators.[135,136] Based on the differences mentioned above, it can be inferred that *P. americana* has evolved some unique physiological mechanisms to deal with the hyperaccumulated Mn compared to other well-studied Mn hyperaccumulators identified outside China.

Rare earth elements hyperaccumulators

The only two REEs hyperaccumulators reported so far in China, *i.e. D. dichotoma* (synonym: *Dicranopteris linearis*) and *Pronephrium simplex*, are fern species.[137-139] In contrast, the two currently known REEs hyperaccumulators outside China are woody species.[140,141] *Dicranopteris linearis* growing in a REEs mining area located in Fujian Province accumulates up to 3045 mg kg^{-1} REEs.[138] Remarkably, hyperaccumulation of REEs also occurs in *D. linearis* leaves collected from a non-mining area located in Guangxi Province (1914 mg kg^{-1}), where REEs concentrations in the associated soils were ~15 mg kg^{-1}. Different *D. linearis* populations from mining and non-mining areas located in southern Jiangxi Province also hyperaccumulated REEs.[142] Thus, REEs hyperaccumulation appears to be a constitutive trait in *D. linearis*, although there are variations in REEs accumulation even between metallicolous populations of *D. linearis*.[142] Rare earth elements hyperaccumulation by this species under laboratory conditions has not yet been recorded.[143] Unlike *D. linearis*, the REEs hyperaccumulator status of *P. simplex* was verified in a field survey as well as in the laboratory.[139] These two species both preferentially accumulated light REEs. For example, the most abundant REEs in pinnae of *D. linearis* were lanthanum (La), cerium (Ce) and neodymium (Nd).[138]

Only a few studies have been performed to address the mechanisms regarding REEs tolerance or accumulation in *D. linearis*. Most of these studies have been focused on the metallicolous populations from southern Jiangxi Province, although little (if any) evidence has shown any superior tolerance or accumulation capability of these populations. Electron microscope and X-ray microanalyses showed that REEs were present in the cell wall, intercellular space, plasmalemma, vesicle and vacuole of the rhizoid endodermis and stele cell of *D. linearis*, but not in the Casparian strip of the fern's adventitious rhizoid.[143] For the REEs accumulated in the pinnae of *D. linearis*, the majority (> 80%) was associated with the cell wall[143] and ~8% was present in chloroplast.[144] There is also evidence that REEs-binding peptides are involved in detoxification of REEs in the pinnae of *D. linearis*.[145] Little evidence for the important role of vacuolar sequestration in REEs detoxification in *D. linearis* is available.

Selenium hyperaccumulators

The only Se hyperaccumulator identified so far in China is *Cardamine hupingshanensis*, which is a recently discovered plant species and has also been called *Cardamine enshiensis* or *Cardamine violifolia* in the literature.[146-148] This species has a noticeable ability to accumulate Se, with up to 1965 mg kg^{-1} Se in the shoot and 4414 mg kg^{-1} Se in its root, when growing in Se-enriched soils (10–70 mg kg^{-1} Se).[147]

Over 70% of the total Se accumulated in the tissues (including root, stem and leaf) of *C. hupingshanensis* is present in the form of selenocysteine (SeCys)[147]. This is remarkable, given that the two other well-studied Se hyperaccumulators (*i.e.*

Astragalus bisulcatus and *Stanleya pinnata*) outside China were found to accumulate Se in their tissues mainly in the form of methylselenocysteine (approximately 70%).[149,150] The rhizosphere of *C. hupingshanensis* harbors a Se-tolerant bacterium that can transfer Se (IV) into SeCys, indicating an important role of rhizosphere bacteria in Se hyperaccumulation by this plant species.[151] In contrast, an endophytic fungus that produces elemental Se was shown to be potentially involved in Se hyperaccumulation in *A. bisulcatus*.[150]

SUMMARY AND OUTLOOK

In summary, significant progress has been made in China during the past 20 years in the discovery of new hyperaccumulator plants and in understanding the mechanisms involved in their tolerance, detoxification and accumulation abilities. Specifically, the major findings can be summarized as follows: (*i*) co-hyperaccumulation of Cd and Zn is not confined to the Brassicaceae family; (*ii*) Mn hyperaccumulation occurs in not only woody species but also herbaceous species; (*iii*) apart from woody plants, ferns are also able to hyperaccumulate REEs; (*iv*) phytic acid was identified as a novel root exudate that can efficiently solubilize As in the rhizosphere of *P. vittata*; (*v*) arsenate reductase plays a major role in the reduction of arsenate in the rhizoid of *P. vittata*; (*vi*) in contrast to *A. halleri* and *N. caerulescens*, the Cd hyperaccumulator *S. alfredii* translocates Cd at least partly via Ca transporters and preferentially allocates the hyperaccumulated Cd in parenchyma cell rather than in the epidermis of its leaf; (*vii*) unlike other Mn hyperaccumulators, the Mn hyperaccumulated in the leaf of *P. americana* is preferentially distributed in epidermal cell and is present predominantly in the form of Mn oxalate; and (*viii*) *PvTIP4;1* was identified as the first As(III) transporter encoding gene from *P. vittata*.

The experimental data published in Chinese language journals did not appear to reveal any mechanisms that were different from those reported in English language journals. This situation is easy to understand, given that a majority of researchers in China have been willing to publish their data in English language journals. However, the relevant references in Chinese language journals have important information that should be kept in mind when reading references about hyperaccumulator plants: (*i*) *C. enshiensis* and *C. hupingshanensis* are synonyms for *C. violifolia*; (*ii*) *D. linearis* is a synonym for *D. dichotoma*; (*iii*) *P. acinosa* is a synonym for *P. americana*; (*iv*) the shoot Cu concentration of *C. communis* growing in the natural habitat can be > 1000 mg kg^{-1}; and (*v*) two *ZIPs* were cloned from *S. alfredii* and were demonstrated to be involved in Cd/Zn hyperaccumulation by this species.

Despite the substantial progress outlined above, more effort is needed to fully understand hyperaccumulator plants from China. The number of angiosperms in China (> 30 000 species or ~10% of the total number of described angiosperms in the world),[152] in combination with the range of metalliferous habitats, is indicative of the potential for discovering more hyperaccumulator plants in China in the coming decades. This may be especially the case for Ni hyperaccumulator plant species, which evolve on Ni-rich ultramafic (serpentine) soils.[19] The ultramafic outcrops in China cover an area of > 4500 km^2,[153] comparable to that in New Caledonia (a hotspot of Ni-hyperaccumulator plants)[154]. But surprisingly, to date no Ni hyperaccumulator species have been discovered in China, although nearly 400 plant species outside China were reported as Ni hyperaccumulators.[11,19] An explanation is that so far only one study was conducted in China to determine Ni concentrations in plants (42 species) growing in ultramafic soils.[155]

The potential of whole genome sequencing in improving our understanding of the complex hyperaccumulation phenomenon was highlighted nearly 10 years ago,[156] but the molecular biology of hyperaccumulator plants from China remains incompletely known. The knowledge about genetics of hyperaccumulators from China generally lagged behind what was already known for other hyperaccumulators outside China.[156] This is especially the case for Cd/Zn hyperaccumulators (*A. halleri* and *N. caerulescens vs S. alfredii*). Technological advances have enabled whole genome sequencing at a significantly lower cost,[157] providing a strong incentive for researchers in China to sequence the genomes of native hyperaccumulator plants. In particular, coupling whole genome sequencing to complementary techniques (*e.g.* transcriptomics, proteomics and metabolomics) will yield substantial explanatory power.

Synchrotron-based techniques (X-ray fluorescence microscopy and X-ray absorption spectroscopy) can also play an important role in deciphering the hyperaccumulation phenomenon when used to assess physiological changes in response to trace element dosing or changes in gene expression.[158] Access to several national synchrotron facilities will make China well-placed globally to advance this field.

Supporting Information

One figure showing the number of articles published each year between 1997–2016 focusing on hyperaccumulators of metal(loid)s or REEs, and one table showing performance characteristics of the hyperaccumulator plants that were reported by researchers working in China during 1997–2016.

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FIGURE AND TABLE CAPTIONS

Figure 1. The hyperaccumulators *Elsholtzia splendens*, *Phytolacca americana*, *Pteris vittata*, *Sedum alfredii* and *Sedum plumbizincicola* growing in their natural habitats (A-E); as well as in field-scale phytoextraction trials (F-J). The images of *P. americana* and *P. vittata* were kindly provided by Dr. Sheng-guo Xue (Central South University) and Dr. Mei Lei (Institute of Geographic Sciences and Natural Resources Research, CAS), respectively.

Figure 2. Diagram showing mechanisms involved in As hyperaccumulation by *P. vittata*. The diagram was drawn based largely on the findings of the references summarized in this review. Solid and dotted coloured lines indicate dominant and minor transport pathways, respectively.

Figure 3. Diagram showing major processes for Cd and Zn hyperaccumulation in *S. alfredii* (End Point Control Mechanism). Shoot accumulation capacity and root absorption potential are two end points regulating Cd and Zn uptake-distribution-storage to shoot tissues. Specific Cd and Zn transport systems including long-distance (xylem) and short distance (cellular or subcellular) played central roles in mediating metal hyperaccumulation. Solid and dotted coloured lines indicate dominant and minor transport pathways, respectively.

Table 1. General information of the plant species that were reported by researchers working in China during 1997–2016 to be able to hyperaccumulate metal(loid)s or rare earth elements (REEs) under both field conditions (*i.e.* in their natural habitats) and laboratory conditions (*i.e.* in confirmatory experiments).

Figure 1

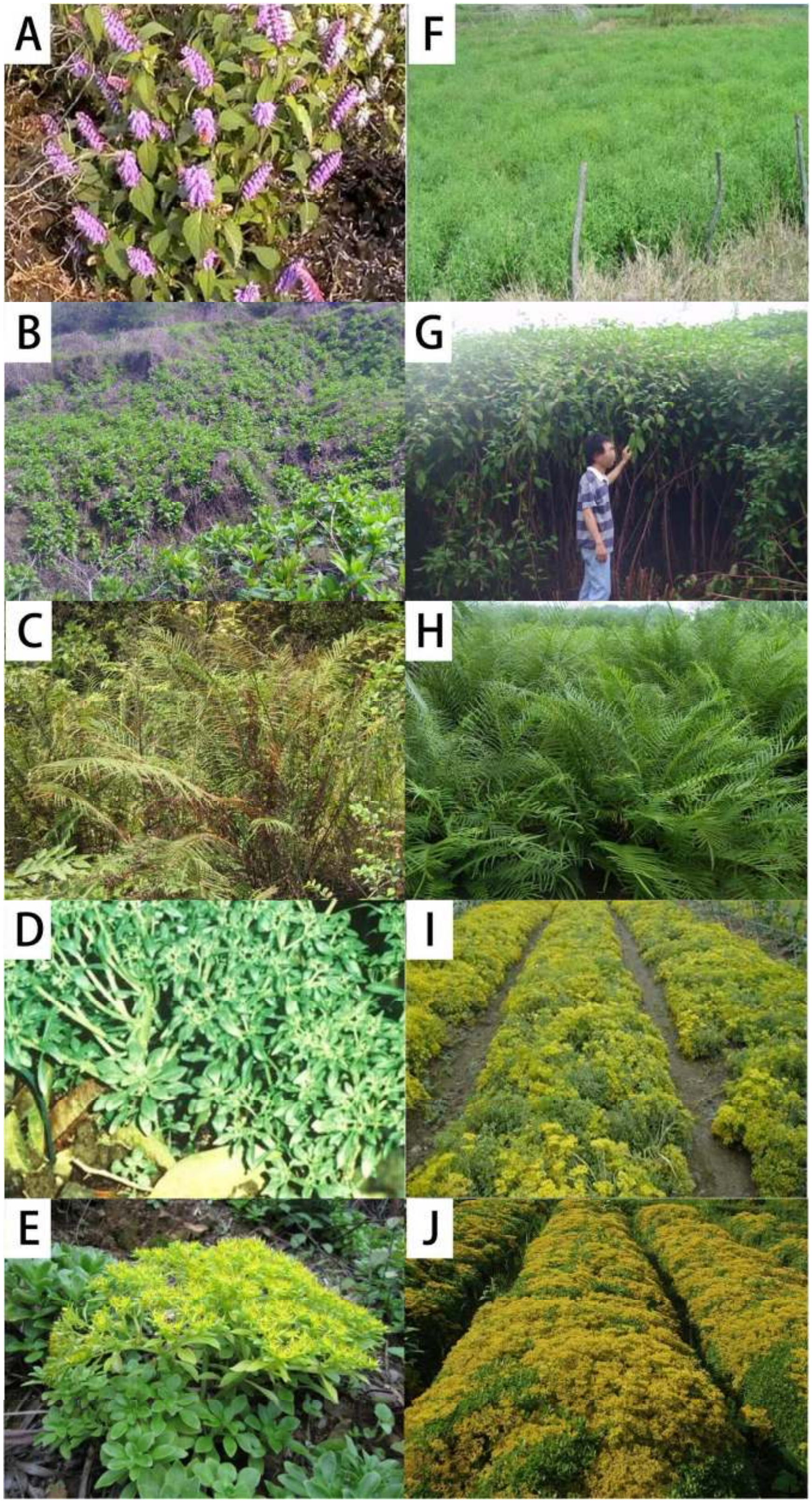

Figure 2

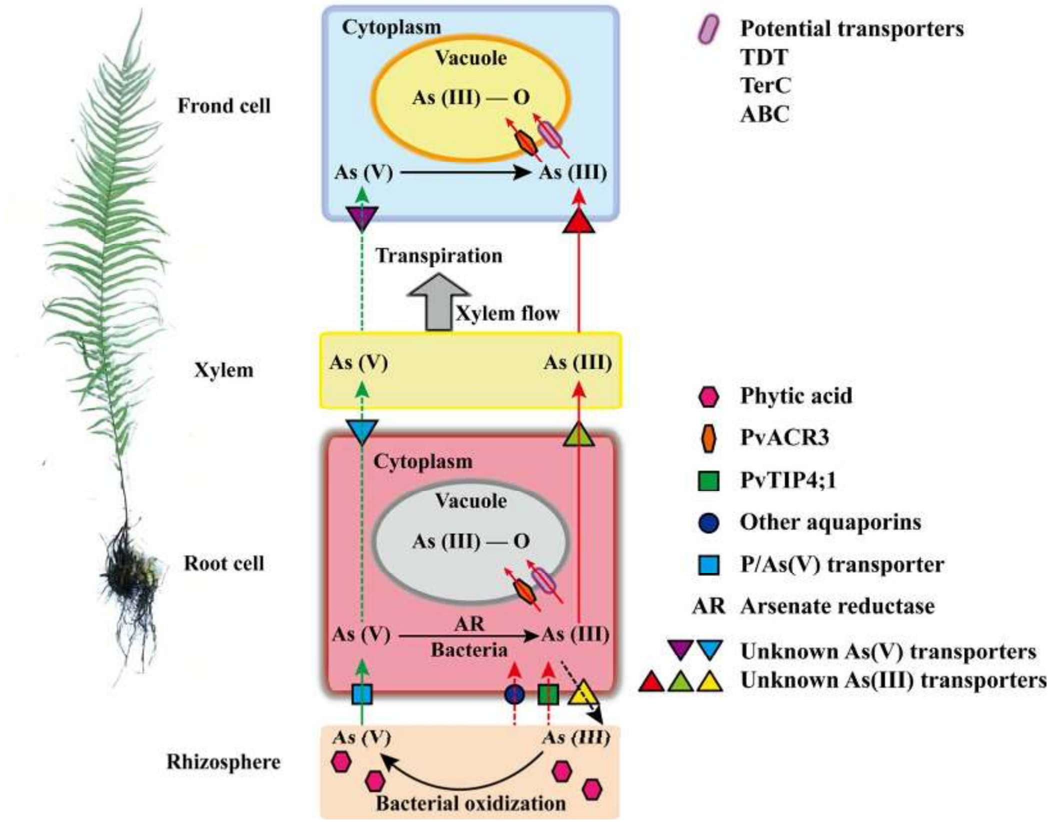
Frond cell
Cytoplasm
Vacuole
As (III) — O
As (V)
As (III)
Transpiration
Xylem flow
Xylem
As (V)
As (III)
Root cell
Cytoplasm
Vacuole
As (III) — O
As (V)
AR
Bacteria
As (III)
Rhizosphere
As (V)
As (III)
Bacterial oxidization
Potential transporters
TDT
TerC
ABC
Phytic acid
PvACR3
PvTIP4;1
Other aquaporins
P/As(V) transporter
AR Arsenate reductase
Unknown As(V) transporters
Unknown As(III) transporters

Figure 3

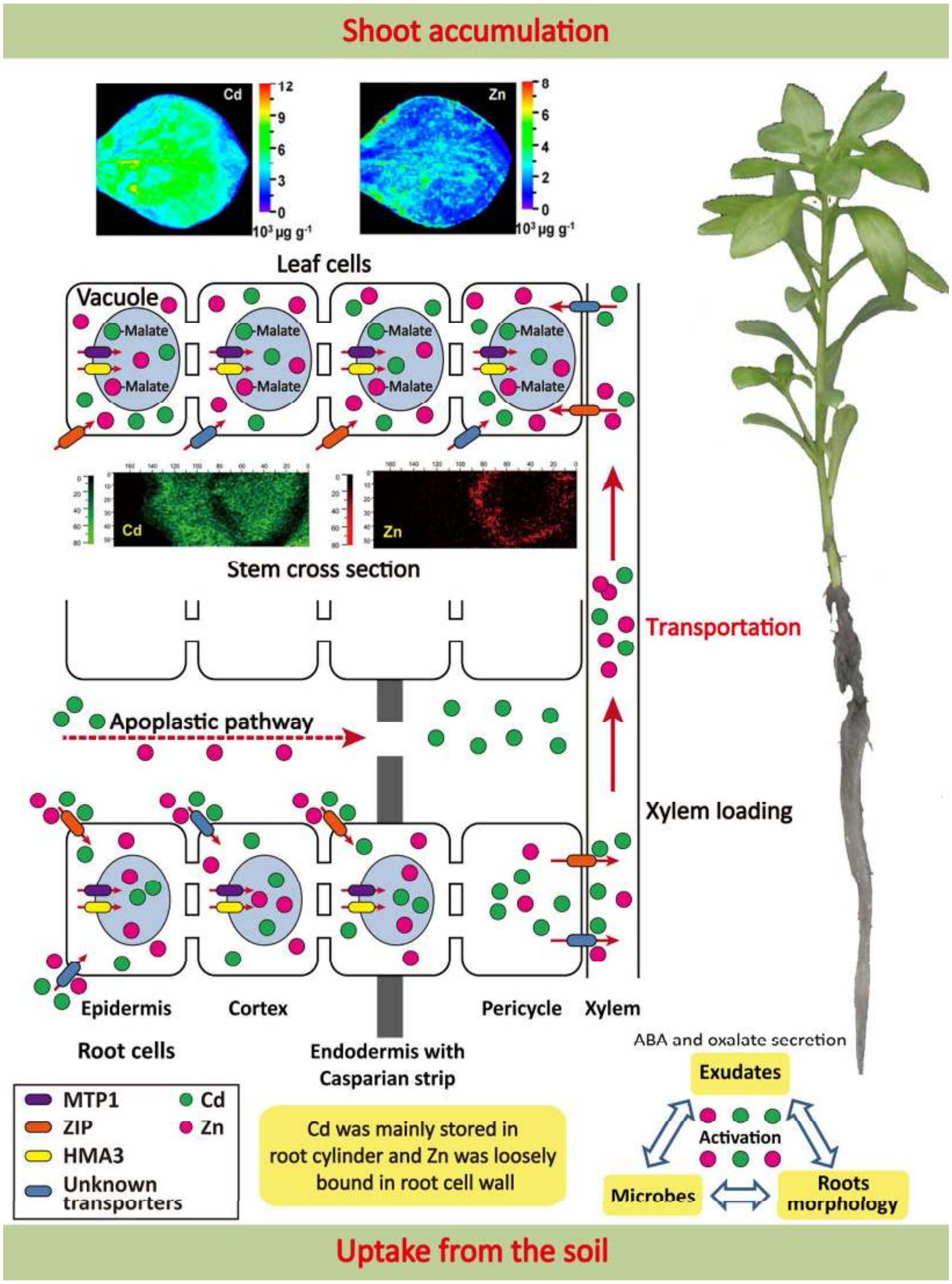
Shoot accumulation
Cd
12
9
6
3
0
10^3 μg g^{-1}
Zn
8
6
4
2
0
10^3 μg g^{-1}
Leaf cells
Vacuole
Malate
Cd
Zn
Stem cross section
Transportation
Apoplastic pathway
Xylem loading
Epidermis
Cortex
Pericycle
Xylem
Root cells
Endodermis with Casparian strip
ABA and oxalate secretion
Exudates
Activation
Microbes
Roots morphology
MTP1
ZIP
HMA3
Unknown transporters
● Cd
● Zn
Cd was mainly stored in root cylinder and Zn was loosely bound in root cell wall
Uptake from the soil

Table 1

plant species	plant family	element	location[&]	distribution	growth habit	obligate/facultative	refs
Pteris cretica	Pteridaceae	As	Nandan, Guangxi	Southern China	Perennial fern up to 0.5-0.7 m tall	Facultative	159 160
Pteris fauriei	Pteridaceae	As	Binyang, Guangxi	Southern China	Perennial fern up to 0.5-0.9 m tall	Facultative	160
Pteris oshimensis	Pteridaceae	As	Nandan, Guangxi	Southern China	Perennial fern up to 0.5-0.8 m tall	Facultative	159 160
Pteris vittata	Pteridaceae	As	Shimen, Hunan	Southern China	Perennial fern up to 0.4-0.6 m tall	Facultative	20
Arabis paniculata	Brassicaceae	Cd	Lanping, Yunnan	Guizhou and Yunan	Biennial herb up to 0.3-0.6 m tall	Facultative	161
Centella asiatica	Apiaceae	Cd	Rongan, Guangxi	Southern China	Perennial herb with creeping stolons	Facultative	162
Phytolacca americana	Phytolaccaceae	Cd	Jishou, Hunan	Almost across China	Perennial herb up to 1-2 m tall	Facultative	163
Picris divaricata	Asteraceae	Cd	Lanping, Yunnan	Tibet and Yunan	Biennial herb 0.1-0.5 m tall	Facultative	164
Potentilla griffithii	Rosaceae	Cd	Lanping, Yunnan	Southwest China	Perennial herb up to 0.1-0.6 m tall	Facultative	165
Sedum alfredii	Crassulaceae	Cd	Quzhou, Zhejiang	Southern China	Perennial herb up to 0.1-0.2 m tall	Facultative	58
Sedum plumbizincicola	Crassulaceae	Cd	Chunan, Zhejiang	Zhejiang	Perennial herb up to 0.35 m tall	Obligate	102 166
Siegesbeckia orientalis	Asteraceae	Cd	Huidong, Sichuan	Southern China	Annual herb up to 0.3-1 m tall	Facultative	167
Viola baoshanensis	Violaceae	Cd	Guiyang, Hunan	Hunan	Perennial herb up	Obligate	168

					to 0.1 m tall		
Arabis paniculata	Brassicaceae	Pb	Lanping, Yunnan	Guizhou and Yunan	Biennial herb up to 0.3-0.6 m tall	Facultative	161
Isache globosa	Gramineae	Pb	Liancheng, Fujian	Almost across China	Perennial grass up to 0.3-0.6 m tall	Facultative	169
Pogonatherum crinitum	Gramineae	Pb	Youxi, Fujian	Southern China	Perennial grass up to 0.1-0.3 m tall	Facultative	169
Celosia argentea	Amaranthaceae	Mn	Daxin, Guangxi	Almost across China	Annual herb up to 0.3-1 m tall	Facultative	170
Phytolacca americana	Phytolaccaceae	Mn	Xiangtan, Hunan	Almost across China	Perennial herb up to 1-2 m tall	Facultative	128
Polygonum lapathifolium	Polygonaceae	Mn	Babu, Guangxi	Almost across China	Annual herb up to 0.9 m tall	Facultative	171
Polygonum pubescens	Polygonaceae	Mn	Quanzhou, Guangxi	Almost across China	Annual herb up to 0.6-0.9 m tall	Facultative	172
Schima superba	Theaceae	Mn	Pingle, Guangxi	Southeast China	Perennial tree up to 25 m tall	Facultative	173 174
Pronephrium simplex	Thelypteridaceae	REEs	Nanjing, Fujian	Southeast China	Perennial fern up to 0.3-0.4 m tall	Facultative	139
Arabis paniculata	Brassicaceae	Zn	Lanping, Yunnan	Guizhou and Yunan	Biennial herb up to 0.3-0.6 m tall	Facultative	161
Corydalis davidii	Fumariaceae	Zn	Hezhang, Guizhou	Southwest China	Perennial fern up to 0.2-1.0 m tall	Facultative	175
Picris divaricata	Asteraceae	Zn	Lanping, Yunnan	Tibet and Yunan	Biennial herb 0.1-0.5 m tall	Facultative	164
Potentilla griffithii	Rosaceae	Zn	Lanping, Yunnan	Southwest China	Perennial herb 0.1-0.6 m tall	Facultative	176

Sedum alfredii	Crassulaceae	Zn	Quzhou, Zhejiang	Southern China	Perennial herb up to 0.1-0.2 m tall	Facultative	62 91
Sedum plumbizincicola	Crassulaceae	Zn	Chunan, Zhejiang	Zhejiang	Perennial herb up to 0.35 m tall	Obligate	102 166
Viola baoshanensis	Violaceae	Zn	Guiyang, Hunan	Hunan	Perennial herb up to 0.1 m tall	Obligate	177

[&] The locality from where hyperaccumulation was recorded.