

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

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Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.8b01060 • Publication Date (Web): 01 Oct 2018

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

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32 **ABSTRACT**

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

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54 **TABLE OF CONTENTS (TOC)/ABSTRACT ART**

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67 INTRODUCTION

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

89 achieved by phytoextraction within a reasonable time frame, which is especially true
90 for slightly or moderately contaminated soils.

91

92 **DEFINING HYPERACCUMULATION STATUS AND POTENTIAL** 93 **PITFALLS**

94 Criteria for defining hyperaccumulation of trace elements have been proposed by
95 Reeves,⁹ Baker and Whiting,¹⁰ and by Van der Ent *et al.*¹¹ In this review, we
96 followed this terminology, *i.e.* a plant that has in excess of the following foliar
97 concentrations (dry weight): 100 mg kg⁻¹ for Cd, selenium (Se) and thallium (Tl);
98 1000 mg kg⁻¹ for As, chromium (Cr), cobalt (Co), copper (Cu), nickel (Ni) and REEs;
99 3000 mg kg⁻¹ for zinc (Zn), and 10 000 mg kg⁻¹ for manganese (Mn) in at least one
100 specimen growing in its habitat while having the ability to complete its life cycle
101 successfully. Although these values may appear arbitrary, they have proved useful
102 for distinguishing abnormal from normal accumulation of metal(loid)s or REEs in
103 plants.^{12,13} This definition emphasized the fact that a plant should be hypertolerant to
104 the element in question and survive without major toxicity symptoms to reproduce
105 successfully. As such, work done solely on dosing plants in solution cultures or
106 spiked soils where plants develop obvious toxicity due to breakdown of the metal
107 regulatory network, but seemingly ‘hyperaccumulate’ are not confirmation of
108 hyperaccumulation status.^{14,15} Although a minimum concentration is needed to
109 achieve uptake, the key characteristics of hyperaccumulator plants are their highly
110 efficient uptake behavior and a non-linear dose response.^{14,15} Provided dose levels

111 are kept low (*e.g.* < 10 μM Co, Mn, Ni or Zn), hydroponic experiment is an
112 important tool to identify genuine hyperaccumulators by confirming hypertolerance,
113 hyperaccumulation in the shoot, high bioconcentration factor and high root-to-shoot
114 metal translocation.^{11,16} Indeed, the other two more strict criteria were proposed to
115 screen out those genuine hyperaccumulators: (i) bioconcentration factor (*i.e.* the
116 shoot-to-soil ratio of element concentration) is > 1; and (ii) translocation factor (*i.e.*
117 the shoot-to-root ratio of element concentration) is > 1.¹⁷ Other confounding issues
118 can result from the contamination of foliar samples with extraneous soil or mineral
119 particulates, an issue arising especially at mining and smelter sites from dust
120 deposition.¹¹ Submerged (aquatic) plant species were not taken into account in this
121 review, due to the risk of external contamination, which is exceedingly difficult to
122 avoid.

123

124 **OVERVIEW OF HYPERACCUMULATOR PLANT SPECIES FROM CHINA**

125 The hyperaccumulator plant species reported by researchers working in China during
126 1997–2016 under both field conditions (*i.e.* natural habitats) and laboratory
127 conditions (*i.e.* confirmatory experiments) are listed in Table 1. Whilst this overview
128 is intended to be as comprehensive as possible, we do not seek to reach any
129 definitive conclusions about the hyperaccumulation status of any given plant species.
130 For example, *Solanum nigrum* has been reported as a Cd hyperaccumulator,¹⁸ but
131 was not included in Table 1 because Cd hyperaccumulation ($\geq 100 \text{ mg kg}^{-1}$) has not
132 been observed in above-ground tissue collected from natural habitats.

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

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

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153

154 **ARSENIC HYPERACCUMULATORS**

155

156 **The occurrence of As hyperaccumulator plant species in China**

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

160 Arsenic hyperaccumulation is a constitutive trait in *P. vittata*,²¹ although there is
161 variability in As accumulation between *P. vittata* populations (or genotypes).^{22,23}
162 Coincidentally, the studies undertaken in China revealed considerable variations (up to
163 5.2-fold differences) in As accumulation between populations.^{22,23} The experiments
164 performed in China typically lasted for a longer time period than those undertaken
165 outside China (84–160 days vs 30 days).^{20,22-24} It has been observed that the shoot As
166 concentration of a *P. vittata* population from Florida increased rapidly within the first
167 8 weeks after exposure to As and gradually approached a plateau in the following 12
168 weeks.²⁵ Further, non-metallicolous populations of *P. vittata* exhibited higher As
169 accumulation but lower As tolerance than metallicolous accessions.^{22,23}

170

171 **Ecophysiology of As hyperaccumulation**

172 Much like other hyperaccumulator plants, *P. vittata* has typical root exudates (such
173 as oxalic acid) which enhance the solubility of As in its rhizosphere. More
174 importantly, the root is able to exude phytic acid, an exudate that has not yet been
175 observed in the rhizospheres of other hyperaccumulator plants.²⁶ Phytate exudation

176 by *P. vittata* has been induced by As exposure and the addition of exogenous phytate
177 into the rhizosphere of *P. vittata* led to a higher As concentration in the frond.²⁶
178 These observations suggest that *P. vittata* has developed a phytate-based strategy to
179 solubilize As from its rhizosphere, thereby mediating efficient As uptake by the root
180 and As hyperaccumulation in the frond (Figure 2).

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

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

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

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

218 Efflux of As(III) to the external environment is another As detoxification
219 pathway in *P. vittata*.⁴⁴ Initially, weak efflux of As(III) from *P. vittata* rhizoid to the

220 external environment was postulated, because the As(III) in As(V) solutions after
221 being used for growing *P. vittata* accounted for a small portion of the total amount of
222 As(V) added to the solutions.³⁰ However, increasing evidence suggests that the
223 efflux of As(III) from *P. vittata* rhizoid could be strong and that bacterially-mediated
224 As(III) oxidation in the growth medium make it difficult to accurately determine the
225 amount of As(III) effluxed by the rhizoid.^{45,46}

226 The majority (> 90%) of As in xylem sap of *P. vittata* is As(III) regardless of the
227 As species added to the growth medium, suggesting a preferential loading of As(III)
228 into xylem over As(V).³⁰ Additionally, there is evidence that both a high-affinity
229 system and a low-affinity system are responsible for As(III) loading into xylem of *P.*
230 *vittata*, with the former being dominant at low As(III) levels and the latter playing a
231 leading role at high As(III) levels.²⁷ It is notable also that the presence of DNP
232 (2,4-dinitrophenol) inhibited the As(III) xylem loading in *P. vittata* and the inhibitory
233 effect decreased with increasing external As(III) concentrations, indicating a more
234 important role of passive diffusion in the As(III) xylem loading at high As(III)
235 concentrations.²⁷

236 Several lines of evidence suggest that transpiration is involved in
237 rhizoid-to-shoot translocation of As by *P. vittata* (Figure 2).⁴⁷ Firstly, the *P. vittata*
238 population from a moister and warmer habitat (with an annual precipitation of 1736
239 mm and an annual temperature of 22°C) exhibited a higher transpiration efficiency
240 and greater shoot As concentrations compared to that from a drier and cooler habitat
241 (with an annual precipitation of 1426 mm and an annual temperature of 17°C), when

242 they were grown in a culture solution containing 1 (one) mg As L⁻¹. Secondly,
243 inhibition of transpiration by the addition of polyethylene glycol into the As-spiked
244 growth media decreased shoot As concentration in both the two populations. Thirdly,
245 the increment of transpiration by adding K into the As-spiked growth media
246 enhanced shoot As concentration in the population with a lower transpiration
247 efficiency.

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

258

259 **Molecular biology of As hyperaccumulation**

260 Recently, a new *P. vittata* aquaporin gene (*PvTIP4;1*) was identified and shown to be
261 responsible for As(III) uptake (Figure 2).⁵⁰ Heterologous expression in
262 *Saccharomyces cerevisiae* suggested that PvTIP4;1 could act as an As(III) uptake
263 channel, whilst transgenic *Arabidopsis* plants expressing *PvTIP4;1* produced less

264 biomass but accumulated more As than the wild-type plants when they were exposed
265 to As(III). Moreover, there is evidence that PvTIP4;1 was localized on the plasma
266 membrane of *P. vittata* protoplasts and that *PvTIP4;1* transcripts were mainly
267 expressed in the rhizoid of *P. vittata*. It was observed, however, that the expression
268 of *PvTIP4;1* was not induced by As(III).⁵⁰ Such a phenomenon, in combination with
269 the lack of effect of aquaporin inhibitors on As(III) uptake by *P. vittata* rhizoid,⁵¹
270 indicates that the contribution of aquaporins in As(III) uptake by *P. vittata* needs
271 further exploration. Despite this, *PvTIP4;1* is the only one known gene encoding a
272 transporter that mediated As(III) uptake into the rhizoid of *P. vittata*.

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

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

286

287 **CADMIUM HYPERACCUMULATORS**

288

289 **The occurrence of Cd hyperaccumulator plant species in China**

290 In China, at least nine plant species belonging to seven families have been identified
291 as Cd hyperaccumulators (Table 1), with *S. alfredii* (Figure 1) being the most studied
292 species. A population of *S. alfredii* from Quzhou was first shown to hyperaccumulate
293 Cd in 2004.⁵⁸ Another Cd hyperaccumulator belonging to the genus *Sedum* (*i.e.* *S.*
294 *plumbizincicola*, Figure 1), found in Chunan and Quzhou in 2006, appears to be
295 morphologically similar to *S. alfredii*.⁵⁹ However, nrDNA internal transcribed spacer
296 sequence analysis has shown that *S. plumbizincicola* is indeed a taxonomic entity
297 distinct from *S. alfredii*.⁶⁰

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

304

305 **Ecophysiology of Cd hyperaccumulation**

306 Cadmium bioavailability in the rhizosphere of the hyperaccumulating population of
307 *S. alfredii* was shown to be higher than that for the non-hyperaccumulating

308 population.^{65,66} The former was reported to be able to exude more dissolved organic
309 matter into its rhizosphere than the non-hyperaccumulating population (Figure 3).⁶⁷
310 Increasing dissolved organic matter correlated with the decrease of pH in the
311 rhizosphere (by 0.6–0.8 units), compared to the bulk soil.^{66,68}

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

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

324 Cadmium was found to be distributed preferentially in the root cylinder of the
325 hyperaccumulating population but not in that of the non-hyperaccumulating
326 population.⁷³ There was a 3–5-fold higher Cd concentration in xylem sap of the
327 hyperaccumulating population than that of the non-hyperaccumulating population.⁷³
328 A greatly enhanced root-to-shoot translocation rate of ^{109}Cd was also observed in the
329 hyperaccumulating population compared to that of the non-hyperaccumulating

330 population,⁶⁹ suggesting that enhanced Cd xylem loading rate plays an important
331 role in Cd hyperaccumulation by *S. alfredii*.⁷³

332 Preferential Cd accumulation in the pith and cortex was observed in the stem of
333 the hyperaccumulating population of *S. alfredii* (Figure 3), whereas Cd was
334 restricted to the vascular bundles in the non-hyperaccumulating population.⁷⁴ In the
335 leaf of the hyperaccumulating population, Cd was preferentially distributed in
336 (photosynthetically active) parenchyma cells rather than in (photosynthetically
337 inactive) epidermis.⁷¹ This finding was remarkable, given that the foliar Cd
338 hyperaccumulated by *A. halleri* and *N. caerulescens* was mainly concentrated in the
339 trichomes and epidermal cells, respectively.⁷⁵⁻⁷⁷ Such a seemingly abnormal
340 phenomenon can be explained by the fact that the parenchyma cells of *S. alfredii*
341 consist of large vacuolar cells which could serve as major storage sites for foliar
342 Cd.⁷¹ Moreover, the cellular distribution patterns of Cd in both the stem and leaf of
343 the hyperaccumulating population were very similar to that of Ca but not Zn (Figure
344 3).^{71,78} This phenomenon has not yet been observed in any other Cd
345 hyperaccumulator, suggesting a possibly important role of Ca transporters in Cd
346 hyperaccumulation by *S. alfredii* (Figure 3).⁷¹ On the other hand, Cd in the leaf of
347 the hyperaccumulating population of *S. alfredii* was found to be mainly associated
348 with oxygen ligands (especially malic acid; Figure 3),⁷¹ which is similar to the
349 observations in *A. halleri* and *N. caerulescens*.^{79,80}

350

351 **Molecular biology of Cd hyperaccumulation**

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

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

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

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

383

384 **ZINC HYPERACCUMULATORS**

385

386 **The occurrence of Zn hyperaccumulator plant species in China**

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

392 There is a remarkable difference between the Zn-accumulating abilities of
393 different *S. alfredii* populations. Even for metallicolous populations collected from
394 mining areas with similar Zn levels in soils, the Quzhou population can accumulate >
395 10 000 mg kg⁻¹ Zn in its shoot, whilst the Jinchuantang (Hunan Province) population

396 accumulates $< 2000 \text{ mg kg}^{-1} \text{ Zn}$.⁶⁶ This variation suggests that Zn hyperaccumulation
397 is not a constitutive trait in *S. alfredii*, in contrast to *A. halleri* and *N. caerulescens*
398 which exhibit constitutive Zn hyperaccumulation.^{63,64} Contrary to the Quzhou
399 population, the non-metallicolous population of *S. alfredii* from Hangzhou (Zhejiang
400 Province) is unable to accumulate $> 1000 \text{ mg kg}^{-1} \text{ Zn}$ in its shoot, and the
401 accumulated Zn is mostly allocated within its root.⁹² Thus, the Hangzhou population
402 has been widely adopted to make comparisons with hyperaccumulating populations
403 to reveal the possible mechanisms underlying Zn hyperaccumulation and
404 hypertolerance of this species.

405

406 **Ecophysiology of Zn hyperaccumulation**

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

415 The bioavailability of Zn in the rhizosphere of the hyperaccumulating
416 population of *S. alfredii* was demonstrated to be greatly enhanced compared to that
417 of the non-hyperaccumulating population, which was due largely to a decrease in

418 rhizosphere pH (by 0.6–0.8 units) driven by more dissolved organic matter.⁷⁰
419 Rhizosphere bacteria of the hyperaccumulating population have been isolated and
420 shown to be able to mobilize insoluble Zn by decreasing pH through production of
421 short-chain organic acids.⁹⁶ Some isolated endophytes are also able to solubilize Zn
422 directly from minerals such as ZnCO_3 and $\text{Zn}_3(\text{PO}_4)_2$, implying their positive roles in
423 the root Zn activation.⁹⁶

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

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

439 At the initial stage of Zn exposure, significantly more Zn is allocated in the

440 stem vascular bundle of the hyperaccumulating population of *S. alfredii* compared to
441 that of the non-hyperaccumulating population, indicating a faster root-to-shoot Zn
442 translocation in the hyperaccumulating population through the vascular bundle
443 (Figure 3).^{100,101} As the exposure time progressed, Zn accumulation in the vascular
444 bundle tended to level off towards saturation, yet that in the stem epidermis of the
445 hyperaccumulating population increased rapidly, leading to high concentrations of
446 Zn in both the vascular bundle and the epidermis. Similarly, in the stem of *S.*
447 *plumbizincicola*, Zn was mainly accumulated in the epidermis and in the vascular
448 bundle and neighbouring parenchyma cell.¹⁰²

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

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

460

461 **Molecular biology of Zn hyperaccumulation**

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

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

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

486

487 **HYPERACCUMULATORS FOR OTHER ELEMENTS**

488

489 **Copper hyperaccumulators**

490 *Commelina communis* growing in Cu mining wastelands located in Tongling, Anhui
491 Province has been found to accumulate 731 (range 369–831) and 547 (429–587) mg
492 kg⁻¹ Cu in its stem and leaf, respectively.¹¹⁶ Based on these field data, *C. communis*
493 was considered initially as a Cu hyperaccumulator. Note, however, that a subsequent
494 field survey showed that the average concentration of Cu in the shoot of a *C.*
495 *communis* population from ‘Tonglvshan’ (Hubei Province) was 1224 mg kg⁻¹.¹¹⁷ This
496 population was then found to grow well in solutions containing up to 51 mg Cu L⁻¹
497 and to be able to accumulate ~540 and 1300 mg kg⁻¹ Cu in leaf and stem,
498 respectively.¹¹⁸ Copper hyperaccumulation has also been observed in another *C.*
499 *communis* population growing in the Baoshan mine located in Tongling.¹¹⁹ Low
500 shoot Cu concentrations were recorded for *C. communis* populations from both
501 contaminated and uncontaminated sites. For example, concentrations of Cu in the
502 stem and leaf of a *C. communis* population growing in Tangshan Cu mine (Jiangsu
503 Province) were only 40 and 28 mg kg⁻¹, respectively.¹²⁰

504 Copper hyperaccumulation in *Elsholtzia splendens* (Figure 1) was originally
505 observed in an old Cu mine located in Zhuji (Zhejiang Province).¹²¹ In a subsequent

506 study, the Zhuji population of *E. splendens* was found to accumulate $\sim 90 \text{ mg kg}^{-1}$ in
507 its leaf after growing for 24 days in a nutrient solution containing $6.36 \text{ mg Cu L}^{-1}$
508 (the highest Cu concentration tested that did not induce toxicity symptom or biomass
509 reduction).¹²² There have been few, if any, reports of Cu hyperaccumulation in other
510 populations of *E. splendens*. In contrast, at least four populations growing in Cu
511 mines located in Anhui, Hubei and Jiangsu Provinces were found to accumulate <
512 500 mg kg^{-1} Cu in their stems and leaves.^{117,123}

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

521

522 **Manganese hyperaccumulators**

523 The first Mn hyperaccumulator discovered in China is *P. americana* (synonym of *P.*
524 *acinoso*, Figure 1),¹²⁸⁻¹³⁰ which is the first known herbaceous Mn hyperaccumulator
525 globally.¹³¹ It can accumulate up to $14\,477 \text{ mg kg}^{-1}$ Mn in its leaf when growing
526 naturally at a Mn mine located in Xiangtan, Hunan Province.¹²⁸⁻¹³⁰ Most work has
527 been confined to *P. americana*, although four other Mn hyperaccumulators have

528 been reported in China (Tables 1 and S1).

529 There are several lines of evidence that transpiration plays an important role in
530 root-to-shoot translocation of Mn in *P. americana* and distribution of Mn in the
531 leaf.¹³² Firstly, concentrations of Mn in a mature leaf of *P. americana* decreased by
532 ~90% when its transpiration was inhibited by wrapping the leaf with a transparent
533 plastic bag. Secondly, Mn concentrations were higher in mature leaves with larger
534 leaf surface area (higher transpiration rate) than in young leaves. Thirdly, the
535 concentration of Mn in the marginal part (the end of transpiration stream) of a leaf
536 was greater than that in the midrib.

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

550 well-studied Mn hyperaccumulators identified outside China.

551

552 **Rare earth elements hyperaccumulators**

553 The only two REEs hyperaccumulators reported so far in China, *i.e.* *D. dichotoma*
554 (synonym: *Dicranopteris linearis*) and *Pronephrium simplex*, are fern species.¹³⁷⁻¹³⁹

555 In contrast, the two currently known REEs hyperaccumulators outside China are
556 woody species.^{140,141} *Dicranopteris linearis* growing in a REEs mining area located
557 in Fujian Province accumulates up to 3045 mg kg⁻¹ REEs.¹³⁸ Remarkably,
558 hyperaccumulation of REEs also occurs in *D. linearis* leaves collected from a
559 non-mining area located in Guangxi Province (1914 mg kg⁻¹), where REEs
560 concentrations in the associated soils were ~15 mg kg⁻¹. Different *D. linearis*
561 populations from mining and non-mining areas located in southern Jiangxi Province
562 also hyperaccumulated REEs.¹⁴² Thus, REEs hyperaccumulation appears to be a
563 constitutive trait in *D. linearis*, although there are variations in REEs accumulation
564 even between metalicolous populations of *D. linearis*.¹⁴² Rare earth elements
565 hyperaccumulation by this species under laboratory conditions has not yet been
566 recorded.¹⁴³ Unlike *D. linearis*, the REEs hyperaccumulator status of *P. simplex* was
567 verified in a field survey as well as in the laboratory.¹³⁹ These two species both
568 preferentially accumulated light REEs. For example, the most abundant REEs in
569 pinnae of *D. linearis* were lanthanum (La), cerium (Ce) and neodymium (Nd).¹³⁸

570 Only a few studies have been performed to address the mechanisms regarding
571 REEs tolerance or accumulation in *D. linearis*. Most of these studies have been

572 focused on the metalicolous populations from southern Jiangxi Province, although
573 little (if any) evidence has shown any superior tolerance or accumulation capability
574 of these populations. Electron microscope and X-ray microanalyses showed that
575 REEs were present in the cell wall, intercellular space, plasmalemma, vesicle and
576 vacuole of the rhizoid endodermis and stele cell of *D. linearis*, but not in the
577 Casparian strip of the fern's adventitious rhizoid.¹⁴³ For the REEs accumulated in the
578 pinnae of *D. linearis*, the majority (> 80%) was associated with the cell wall¹⁴³ and
579 ~8% was present in chloroplast.¹⁴⁴ There is also evidence that REEs-binding
580 peptides are involved in detoxification of REEs in the pinnae of *D. linearis*.¹⁴⁵ Little
581 evidence for the important role of vacuolar sequestration in REEs detoxification in *D.*
582 *linearis* is available.

583

584 **Selenium hyperaccumulators**

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

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

594 *Astragalus bisulcatus* and *Stanleya pinnata*) outside China were found to accumulate
595 Se in their tissues mainly in the form of methylselenocysteine (approximately
596 70%).^{149,150} The rhizosphere of *C. hupingshanensis* harbors a Se-tolerant bacterium
597 that can transfer Se (IV) into SeCys, indicating an important role of rhizosphere
598 bacteria in Se hyperaccumulation by this plant species.¹⁵¹ In contrast, an endophytic
599 fungus that produces elemental Se was shown to be potentially involved in Se
600 hyperaccumulation in *A. bisulcatus*.¹⁵⁰

601

602 **SUMMARY AND OUTLOOK**

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

616 leaf of *P. americana* is preferentially distributed in epidermal cell and is present
617 predominantly in the form of Mn oxalate; and (viii) *PvTIP4;1* was identified as the
618 first As(III) transporter encoding gene from *P. vittata*.

619 The experimental data published in Chinese language journals did not appear to
620 reveal any mechanisms that were different from those reported in English language
621 journals. This situation is easy to understand, given that a majority of researchers in
622 China have been willing to publish their data in English language journals. However,
623 the relevant references in Chinese language journals have important information that
624 should be kept in mind when reading references about hyperaccumulator plants: (i)
625 *C. enshiensis* and *C. hupingshanensis* are synonyms for *C. violifolia*; (ii) *D. linearis*
626 is a synonym for *D. dichotoma*; (iii) *P. acinosa* is a synonym for *P. americana*; (iv)
627 the shoot Cu concentration of *C. communis* growing in the natural habitat can be >
628 1000 mg kg⁻¹; and (v) two ZIPs were cloned from *S. alfredii* and were demonstrated
629 to be involved in Cd/Zn hyperaccumulation by this species.

630 Despite the substantial progress outlined above, more effort is needed to fully
631 understand hyperaccumulator plants from China. The number of angiosperms in
632 China (> 30 000 species or ~10% of the total number of described angiosperms in
633 the world),¹⁵² in combination with the range of metalliferous habitats, is indicative of
634 the potential for discovering more hyperaccumulator plants in China in the coming
635 decades. This may be especially the case for Ni hyperaccumulator plant species,
636 which evolve on Ni-rich ultramafic (serpentine) soils.¹⁹ The ultramafic outcrops in
637 China cover an area of > 4500 km²,¹⁵³ comparable to that in New Caledonia (a

638 hotspot of Ni-hyperaccumulator plants)¹⁵⁴. But surprisingly, to date no Ni
639 hyperaccumulator species have been discovered in China, although nearly 400 plant
640 species outside China were reported as Ni hyperaccumulators.^{11,19} An explanation is
641 that so far only one study was conducted in China to determine Ni concentrations in
642 plants (42 species) growing in ultramafic soils.¹⁵⁵

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

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

660 field.

661

662 **Supporting Information**

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

667

668 **ACKNOWLEDGEMENTS**

669 This work was supported by Research Programs from the Ministry of Science and
670 Technology of China (Nos. 2016YFD0800805 and 2017YFD0800900) and the
671 National Natural Science Foundation of China (Nos. 41622106, 41471257 and
672 31372128). A. van der Ent is the recipient of a Discovery Early Career Researcher
673 Award (DE160100429) from the Australian Research Council.

674

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1251 **FIGURE AND TABLE CAPTIONS**

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1253 **Figure 1.** The hyperaccumulators *Elsholtzia splendens*, *Phytolacca americana*,
1254 *Pteris vittata*, *Sedum alfredii* and *Sedum plumbizincicola* growing in their natural
1255 habitats (A-E); as well as in field-scale phytoextraction trials (F-J). The images of *P.*
1256 *americana* and *P. vittata* were kindly provided by Dr. Sheng-guo Xue (Central South
1257 University) and Dr. Mei Lei (Institute of Geographic Sciences and Natural Resources
1258 Research, CAS), respectively.

1259

1260 **Figure 2.** Diagram showing mechanisms involved in As hyperaccumulation by *P.*
1261 *vittata*. The diagram was drawn based largely on the findings of the references
1262 summarized in this review. Solid and dotted coloured lines indicate dominant and
1263 minor transport pathways, respectively.

1264

1265 **Figure 3.** Diagram showing major processes for Cd and Zn hyperaccumulation in *S.*
1266 *alfredii* (End Point Control Mechanism). Shoot accumulation capacity and root
1267 absorption potential are two end points regulating Cd and Zn
1268 uptake-distribution-storage to shoot tissues. Specific Cd and Zn transport systems
1269 including long-distance (xylem) and short distance (cellular or subcellular) played
1270 central roles in mediating metal hyperaccumulation. Solid and dotted coloured lines
1271 indicate dominant and minor transport pathways, respectively.

1272

1273 **Table 1.** General information of the plant species that were reported by researchers
1274 working in China during 1997–2016 to be able to hyperaccumulate metal(loid)s or
1275 rare earth elements (REEs) under both field conditions (*i.e.* in their natural habitats)
1276 and laboratory conditions (*i.e.* in confirmatory experiments).

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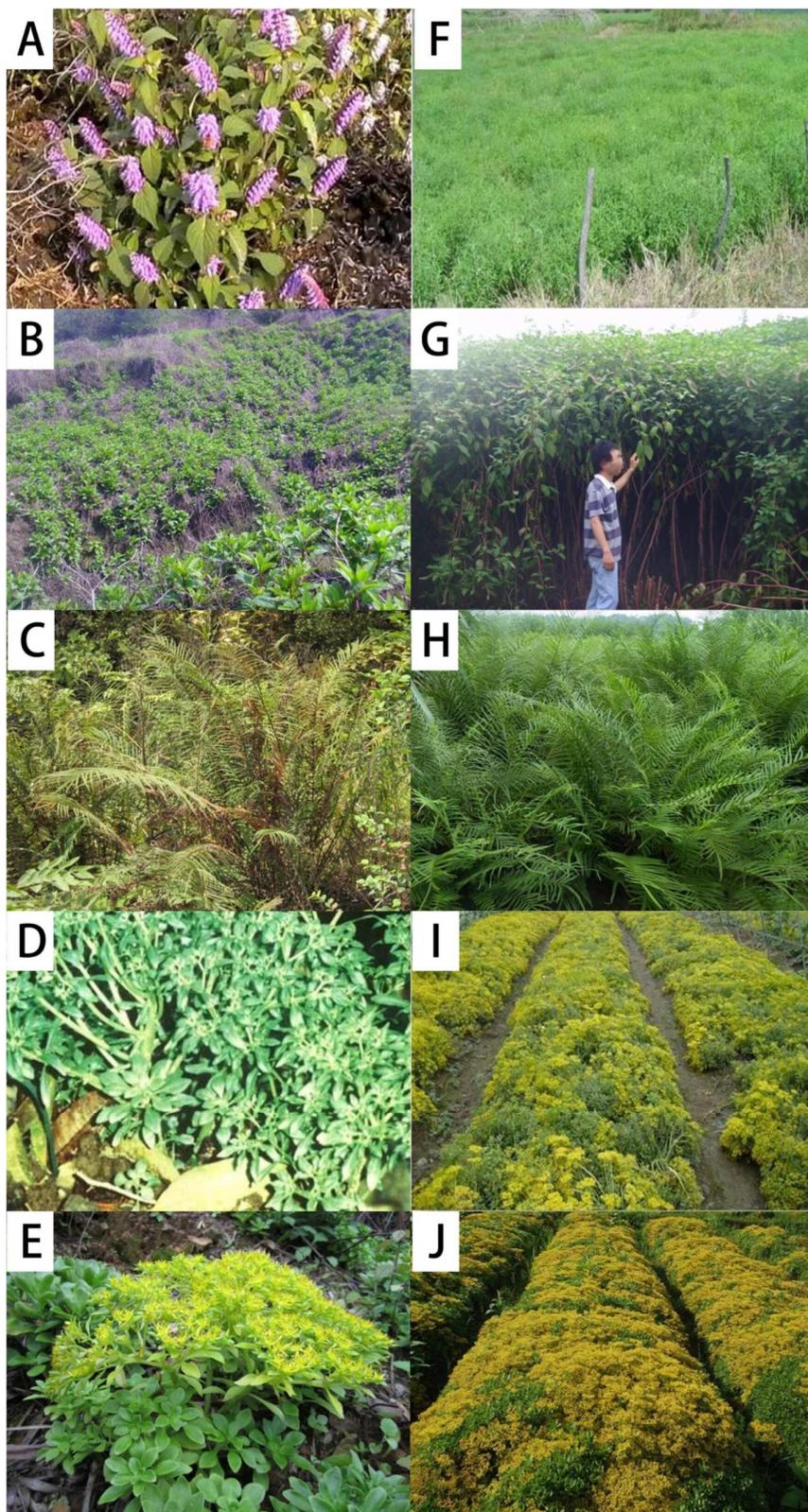
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Figure 1

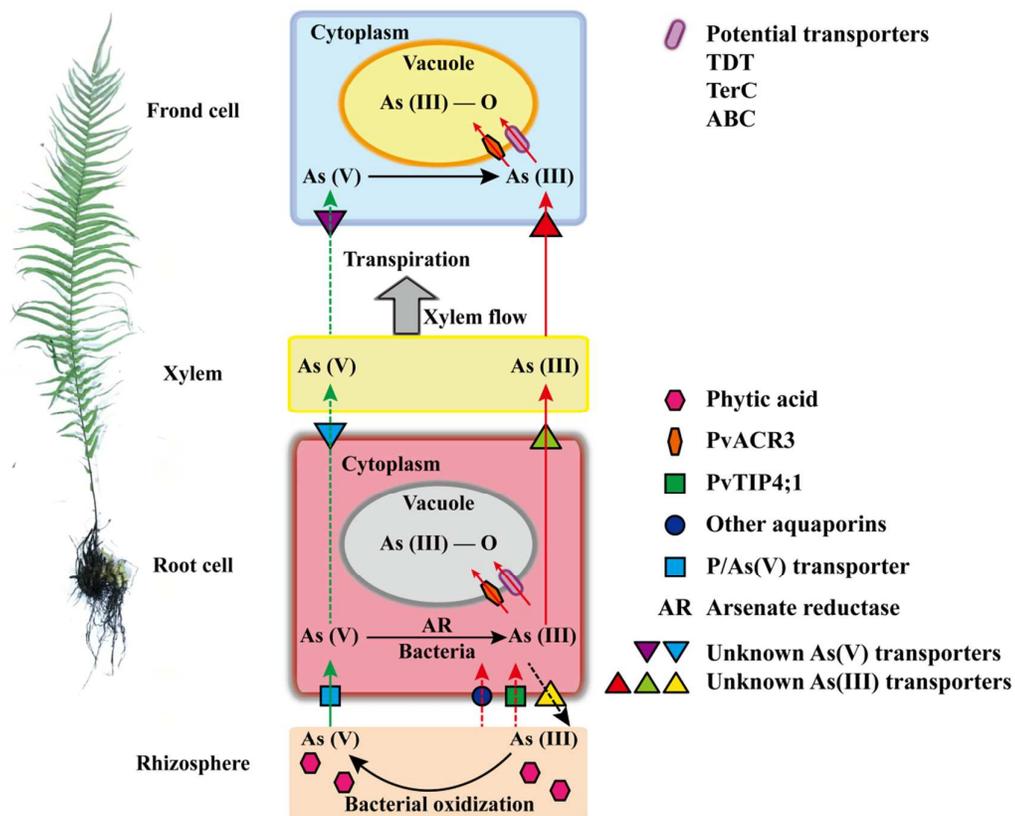


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Figure 2



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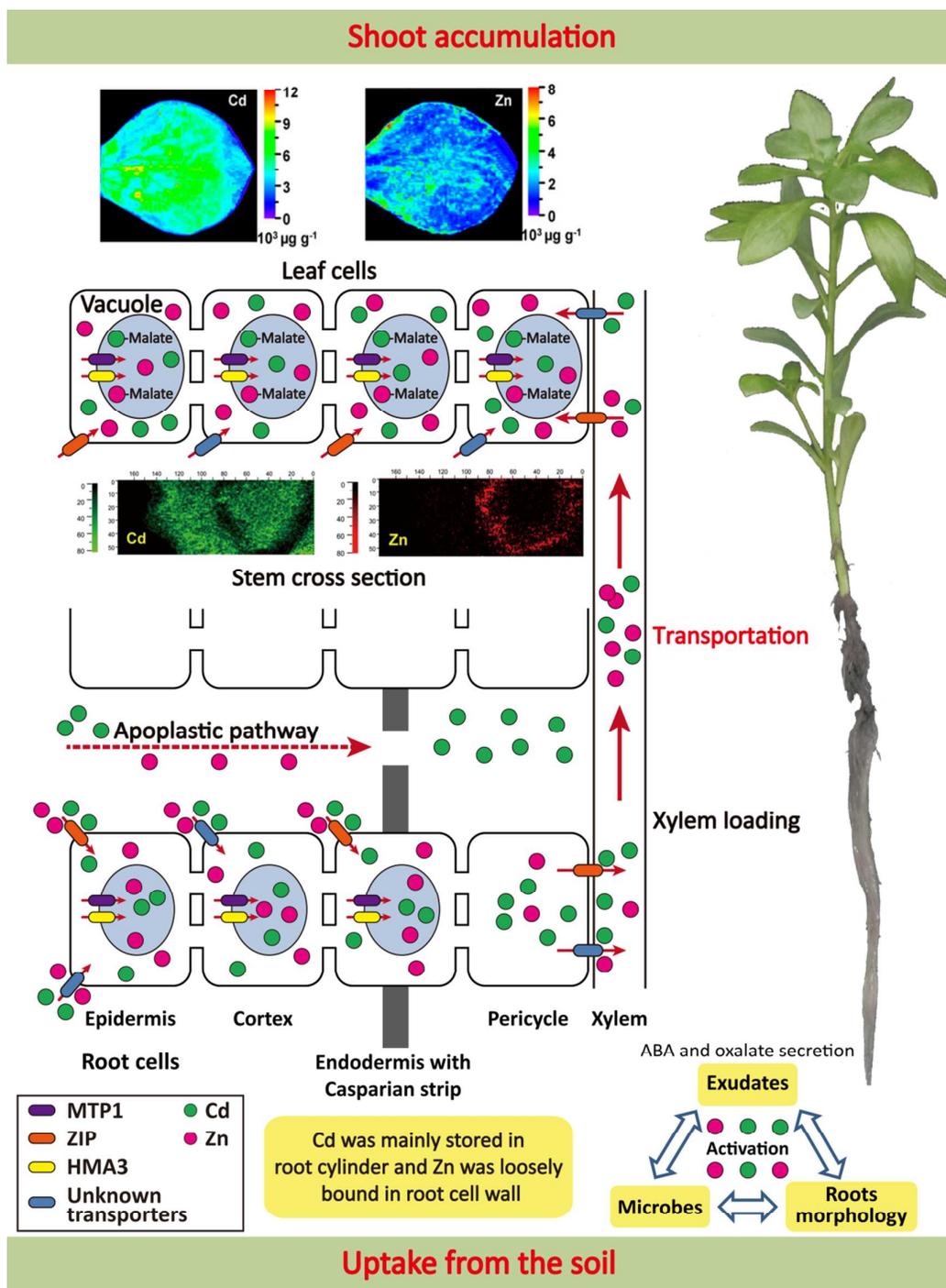
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Figure 3



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Table 1

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

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

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

*The locality from where hyperaccumulation was recorded.