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1 **Mechanisms of cadmium accumulation in plants**

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18 **Mechanisms of cadmium accumulation in plants**

19 **Abstract**

20 Cadmium is a non-essential trace metal, which is highly toxic to nearly all living organisms.
21 Soil pollution causes Cd contamination of crops, thereby rendering plant products responsible
22 for the chronic low level Cd over-exposure of numerous populations in the world. For this
23 reason, Cd accumulation in plants has been studied for about five decades now. The research
24 first focused on the relationships between plant and soil Cd levels, on the factors of the metal
25 availability in soil, as well as the root uptake processes. Cd distribution in plant organs was
26 also investigated, first using a macroscopic and eco-physiological approach, then with the
27 help of molecular biology tools, at both tissue and cell scale. Cadmium has no biological
28 function and hijacks the transport pathways of micronutrients such as Fe, Mn or Zn, in order
29 to enter the plant through the roots and be distributed to all its organs. The study of the genes
30 that control the influx and efflux of the Cd^{2+} ion in the cytosol, vacuoles and vascular tissues
31 has significantly contributed to the understanding of the metal root uptake and of its transfer
32 to the aerial parts. However, the mechanisms responsible for its distribution to the different
33 aboveground tissues and specially to fruits and seeds have yet to be clarified. This review
34 summarizes current knowledge in order to present a detailed overview of Cd transport and
35 storage, from the rhizosphere to the different organs and tissues of the plant.

36

37 **Keywords**

38 Ion transporter; cell wall; chelation; phytochelatin; vacuolar sequestration; xylem sap;
39 phloem; inter-element competition; speciation; organic acid; zinc; iron; silicon; manganese;
40 nitrogen

Cadmium is a transition metal considered as a trace element by geochemists, as its concentration in the upper continental crust - around 0.1 mg kg^{-1} - is well below the limit between trace and major elements (1000 mg kg^{-1}). Cadmium has no known biological function in any organism, except for the marine diatom *Thalassiosira weissflogii*, in which it can replace zinc as cofactor of carbonic anhydrase (Lane *et al.*, 2005). On the contrary, Cd is highly toxic to most living organisms. The metal exerts its toxic effects by competing with essential metals and calcium, as well as inducing oxidative stress through perturbation of the glutathione redox balance. When internalized, it is retained with a biological half-life >30 years in the human body. It accumulates in kidneys and bones and can cause osteoporosis, kidney failure, heart disease and cancer (EFSA, 2012). This makes low-level chronic Cd exposure a serious threat to human health. This is the reason why Cd use has been severely limited by the European REACH regulation (http://ec.europa.eu/growth/sectors/chemicals/reach_en).

Apart from tobacco smoking, food is the main source of human exposure to Cd, through cereals, vegetables, roots and tubers (EFSA, 2012). In these plant products, Cd comes from the soil, through root absorption and translocation to the harvested organs. Most of the soil Cd originates from contamination resulting from human activities. The sources of contamination are atmospheric deposition of industrial and urban emissions, phosphate fertilizers, organic and mineral amendments. In Europe, as atmospheric deposition has decreased since the mid-1960s, Cd from P fertilizer is now the major input into cultivated soils (Six and Smolders, 2014; Sterckeman *et al.*, 2018). The dominant form of the metal in soil is Cd^{2+} . The physico-chemical properties of this ion lead to its accumulation in the soil's ploughed layer by binding to soil solid components. However, this binding is largely reversible (Smolders *et al.*, 1999), making Cd one of most available trace metals for root absorption after desorption into the soil solution (Lin *et al.*, 2016b).

Based on the weekly intake limit recommended by the US Agency for Toxic Substances and Disease Registry (ATSDR, 2012) of $0.7 \mu\text{g Cd per kg of body weight per week}$, most populations of the world would be largely overexposed to this metal through low-level chronic exposure due to food consumption (Clemens *et al.*, 2013). Considering the threshold set by the European Food Safety Authority (EFSA, 2012), which is over 3 fold higher than the one set by ATSDR ($2.5 \mu\text{g Cd kgbw}^{-1} \text{ w}^{-1}$), European populations ($2.04 \mu\text{g Cd kgbw}^{-1} \text{ w}^{-1}$) are on average just below the limit, with some of them being overexposed.

Cadmium chemistry in soil and its accumulation in plants have been studied since the seventies (*e.g.* (Lagerwerff, 1971; John *et al.*, 1972; Haghiri, 1973). The aim of the investigations carried out until now has been to understand the soil supply of Cd to roots as well as the distribution, the toxicity and the tolerance of the metal in the plant organs. Several works have shown that Cd distribution in the plant varies greatly both from one species to another and within a given species, depending on the population or the cultivar (Florijn and Van Beusichem, 1993b; Greger and Lofstedt, 2004; Gonneau *et al.*, 2014; Laporte *et al.*, 2015). This highlights the importance of genetic factors in this distribution. Over the two last decades, numerous publications have addressed the biomolecular processes of Cd accumulation in plants, with a particular effort being made to identify the genes and related proteins responsible for Cd uptake, transport, storage and detoxification in plant tissues.

An underlying application of these works is the creation of cultivars that accumulate less Cd in their consumed parts (Clemens *et al.*, 2013). Another one is the decontamination of soils by phytoextraction, *i.e.* the cultivation of plants accumulating high levels of Cd in their aerial parts, to the point that they would significantly decrease the soil's Cd content. Phytoextraction has been considered with low Cd accumulating and high biomass producing species, such as sunflower (*Helianthus annuus*), maize (*Zea mays*), poplar or willow. An alternative is the use of species which hyperaccumulate and are hypertolerant to metals such as Cd, Ni or Zn

(Chaney *et al.*, 2014; Sterckeman and Puschenreiter, 2018). These wild plants are rare and peculiar, as they are able to grow on metalliferous soils that are toxic to most other species. For this reason, they have been studied extensively to the point of becoming experimental models (Cobbett, 2003; Verbruggen *et al.*, 2009b; Honjo and Kudoh, 2019). These include *Arabidopsis halleri*, *Noccaea caerulescens* (Brassicaceae) or *Sedum alfredii* (Crassulaceae).

We have written this review bearing in mind these underlying applications, which both require an in-depth understanding of the mechanisms controlling Cd flow from soil to plant, in order to be able to either reduce the Cd content in the consumed parts (grain, tubers, leaves) or increase it in the harvested shoots of the plant used to decontaminate the soils. This review thus focuses both on the processes responsible for the transfer of Cd from soil to plant and on those responsible for the distribution of the metal in the different plant parts under non-toxic conditions. The mechanisms of Cd toxicity and tolerance are not addressed as such in this review, but only with regards to their possible contribution to the flow and the distribution of the metal in the plant. For instance, Cd sequestration in cell walls and in vacuoles is a way to reduce Cd toxicity in plants, but it will be regarded here more as a factor controlling Cd distribution and storage among tissues and organs. The reader may refer to other literature reviews regarding the management of Cd toxicity by plants (Moullis, 2010; Lin and Aarts, 2012; Verbruggen *et al.*, 2013; Choppala *et al.*, 2014; Kupper and Andresen, 2016).

I. Overview of the plant Cd accumulation processes

Cadmium exists mainly in the soil as the divalent cation Cd^{2+} . Cadmium concentration as free hydrated ions in soil solutions is in equilibrium with dissolved Cd complexes and Cd adsorbed onto the soil solid phase. By the means of proton excretion, root cells maintain a negative gradient across their plasma membrane, which drives cation absorption, including that of Cd^{2+} . The absorption of Cd^{2+} by root cells reduces the ion concentration at the root

115 surface, generating a concentration gradient, which in turn drives diffusion of Cd^{2+} towards
116 the root. Cadmium is also transported by advection, where the ion moves towards the root at
117 the same speed as the water taken up by the plant for transpiration. However, in the case of
118 Cd, advection is most frequently negligible when compared to diffusion. In addition, Cd^{2+}
119 desorbed from the solid phase replenishes the soil solution as the root depletes it (Figure 1).

120 Some of the Cd ions in the solution adsorb onto the pecto-cellulosic walls of the root cells
121 (root apoplast). Others diffuse through the apoplast until they meet the first apoplastic barrier,
122 i.e. a domain of the extracellular matrix that prevents the diffusion of ions between cells due
123 to deposits of impermeable polymers such as lignin (Casparian strip) or suberin. Depending
124 on the plant species or on the location along the root, the first apoplastic barrier is the
125 exodermis, located just below the root absorptive epidermis in rice for example, or
126 endodermis, delimiting the central cylinder where the root vasculature is located (Figures 2-
127 4). From the apoplast, the ions cross the cell membrane (plasma membrane) using the
128 transport proteins. Under a membrane potential of -120 mV, as usually measured in root cells,
129 Cd^{2+} may accumulate passively by 4 orders of magnitude. Therefore, active transport is not
130 required to drive Cd^{2+} uptake into root cells. Once in the cytosol, Cd ions are complexed by
131 organic ligands. This reduces the concentration of free Cd^{2+} in the cytosol and therefore the
132 metal toxicity. Some of the Cd in the cytosol diffuses towards the xylem, following the
133 plasmodesmata to pass from one cell to another to reach the xylem parenchyma (Figures 2-4).
134 The metal can then be loaded into the xylem vessels by the way of an active transport system,
135 against its electrochemical gradient. Other Cd compounds in the cytosol are distributed to
136 intracellular organelles such as the mitochondria, the Golgi apparatus and mainly the vacuole,
137 through transport proteins. In the vacuole lumen, Cd is mostly chelated by organic ligands.

138 It can therefore be considered that once Cd^{2+} is absorbed into the root symplast, its
139 translocation to the aerial parts is governed by three main processes: sequestration in the root

cells, transport in the symplast to the stele and xylem loading. The competition between these three processes leads most higher plants to accumulate much more Cd in their roots than in their shoots. Nonetheless, some species, such as hyperaccumulating plants, accumulate a higher concentration of the metal in their above ground parts than in their roots.

Once in the xylem sap, Cd^{2+} eventually forms complexes with ligands. These complexes, together with free ions, are transported to the plant aerial parts with the xylem sap flow being driven by transpiration (advection). During this transport, the ions interact with the cell walls of xylem vessels and partly adsorb on them. They also migrate through xylem cell walls to those of the stem and leave cells, under the influence of the transpiration flow. From the apoplast, Cd^{2+} crosses cell membranes *via* transport proteins (Figure 5). Once in the leaf cell cytosol, the metal is generally chelated by organic ligands and migrates from cell to cell. Some of the free ions and of the complexes enter the organelles and particularly the vacuole, where they are stored as chelates. In the shoot, Cd^{2+} is finally distributed in variable amounts according to the cell compartment (wall, cytosol, vacuoles, etc.) and to the tissue (vascular tissues, parenchyma, epidermis...), with a speciation differing with the location and with the plant species.

Although less documented, it is likely that part of the Cd accumulated in the leaves is redistributed to other plant organs *via* the phloem, or even to the roots where Cd could be excreted. The phloem flow also allows Cd transport to the reproductive organs and its accumulation in the seeds. Some works suggest that phloem could be involved in Cd transport before it reaches the leaves, thanks to exchanges from xylem to phloem in the stem.

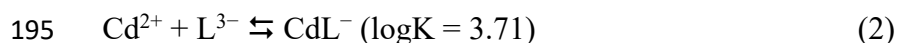
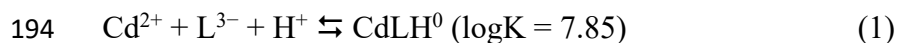
II. Chemistry of Cd in the rhizosphere

Plant roots take up Cd from the surrounding soil solution. The chemistry of Cd in the rhizosphere can usefully be considered as the reactions that control the species and the

concentrations of the metal in solution. These reactions take place in the soil solution and at the surface of the solid phase (Figure 1). With an ionic potential ($IP = Z/r$, the valence divided by the ionic radius in nm) of 21 nm^{-1} , Cd^{2+} is a solvating cation, whose behaviour can be differentiated from that of hydrolysing cations, such as Al^{3+} ($IP = 56 \text{ nm}^{-1}$), and oxyanion-forming cations, such as Mo^{6+} ($IP = 143 \text{ nm}^{-1}$) (Sposito, 1989). Based on this parameter, Cd^{2+} behaves similarly to Ca^{2+} ($IP = 20 \text{ nm}^{-1}$). However, it is differentiated from this cation by its Misono softness parameter (Y , nm), whose value is 0.31 nm (Misono *et al.*, 1967; Sposito, 1989). Values of Y above 0.32 nm correspond to metal ions with high polarizability and low electronegativity, which tend to form covalent chemical bonds, while values below 0.25 nm correspond to ions with low polarizability and high electronegativity tending to form ionic bonds. Cadmium, together with Cu^{2+} , Mn^{2+} , Ni^{2+} and Fe^{2+} fall into the “borderline” zone ($0.25 < Y < 0.32$), while Ca^{2+} has a Y value of 0.16 nm and Hg and Pb have Y values of 0.40 nm. This makes Cd^{2+} chemical behaviour intermediate between that of Ca^{2+} , forming ionic bonds, and those of Pb^{2+} and Hg^{2+} , often involved in covalent bonds.

In the solution of non- or low contaminated soils, Cd concentration is generally below 10 nM, while in Cd polluted soils, it ranges between 10 nM to 200 nM, and can even reach micromolar concentrations under extreme contamination and solubility conditions (Helmke, 1999; Sauvé *et al.*, 2000b; Weng *et al.*, 2002; Nolan *et al.*, 2003; Maxted *et al.*, 2007). The free hydrated ion (Cd^{2+}) is in equilibrium with organic and inorganic complexes. Cd^{2+} reacts with water to form $\text{Cd}(\text{OH})_2$, $\text{Cd}(\text{OH})^+$ and $\text{Cd}(\text{OH})_3^-$ (Gu *et al.*, 2010). In the presence of chloride, particularly in salt affected soils, Cd^{2+} can form soluble chloride complexes, i.e. CdCl^+ , CdCl_2 , CdCl_3^- and CdCl_4^{2-} . Cd^{2+} reacts with sulphate, to form CdSO_4 complexes and, in alkaline soil, with carbon dioxide to form CdCO_3 and CdHCO_3^+ in solution (Alloway, 1995; Helmke, 1999). Free Cd^{2+} is considered to dominate in soil solutions (Degryse *et al.*, 2009; Ren *et al.*, 2015a), although some measurements and modelling have shown that this is

not always the case (Schneider, 2008; Lin *et al.*, 2016b). Indeed, the free hydrated ion can associate to a large extent with soluble humic compounds (Schneider, 2008; Ren *et al.*, 2015a; Ren *et al.*, 2015b), as well as with well-defined molecules such as organic acids (Schneider and Nguyen, 2011). For instance, Lackovic *et al.* (2004) proposed several reactions to model Cd complexation by citrate (noted L):



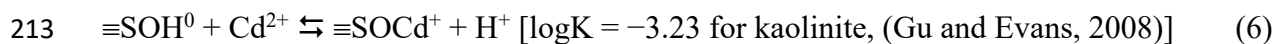
Cadmium competes with other cations, such as Ca^{2+} for complexation with soluble organic ligands (Lin *et al.*, 2016b).

In most soils, the amount of dissolved Cd is much lower than that bound to the solid phase. Cadmium can precipitate as carbonate or phosphate, but soils are generally under-saturated with these minerals (Tye *et al.*, 2003). Even if super-saturated, dissolved organic matter has been reported to inhibit CdCO_3 precipitation (Holm *et al.*, 1996). Therefore, rather than precipitation, adsorption on mineral surfaces and binding to humic substances are the processes which control Cd partitioning between solid and liquid phases (Lofts and Tipping, 1998; Tipping, 1998; Ren *et al.*, 2015b). Indeed, Cd can bind with particulate fulvic or humic acid (R) with a net charge Z, exchanging against protons according to the following equation:



The metal also interacts with the surface sites on minerals, particularly on Fe and Mn hydroxides and clay minerals. Surface complexation reactions on variable charged sites

211 ($\equiv\text{SOH}$), such as those at the edges of clay minerals or of iron hydroxides can be formalized
 212 by:



214 while adsorption on permanent negatively charged sites ($\equiv\text{X}^-$) is



216 However, as the soil matrix is very complex, with numerous constituents having each
 217 different types of surface reactive sites, Cd adsorption is generally modelled using the values
 218 of the distribution coefficient, K_d (L. kg^{-1}), which is the ratio of Cd reversibly adsorbed on the
 219 solid phase divided by metal in solution. K_d ranges from several tens to several thousands
 220 (Sauvé *et al.*, 2000a; Sauvé *et al.*, 2000b). K_d is generally well predicted by a function of soil
 221 organic carbon and pH (Sauvé *et al.*, 2000a; de Meeûs *et al.*, 2002; Degryse *et al.*, 2009), such
 222 that

223 $\log (K_d) = -1.04 + 0.55\text{pH}_{\text{CaCl}_2} + 0.70\log (C_{\text{org}})$ ($n = 123$, $R^2 = 0.72$) (8)

224 proposed by Degryse *et al.* (2009), C_{org} being the organic carbon content (%) and $\text{pH}_{\text{CaCl}_2}$ the
 225 pH of a soil suspension in a 0.01 M CaCl_2 solution. This relationship supports the idea that
 226 organic carbon is the most important reactive fraction of the soil solid phase (Tye *et al.*, 2003;
 227 Ren *et al.*, 2015b) and that Cd sorption increases with organic carbon content of the solid
 228 phase. In addition, the solubility of Cd associated to Fe oxides is affected by the soil redox
 229 potential (Yu *et al.*, 2016). Moreover, in carbonated soils, Cd can sorb at the surface of
 230 calcite, with Cd^{2+} replacing Ca^{2+} . This could lead to an irreversible absorption, as Cd diffuses
 231 through the calcite and makes a solid solution of otavite-calcite (Martin-Garin *et al.*, 2003).

232 As with complexation in solution, Cd sorption is a reversible and competitive process, which
 233 depends on the various cations in solution together with Cd^{2+} . Eqs. 5-8 indicate that protons

compete with Cd^{2+} both for adsorption on the soil minerals and for binding with humic substances phase. Various works have evidenced that Cd sorption increases with pH (Christensen, 1984; Sauvé *et al.*, 2000a). Eq. 8 shows that sorption at pH = 5.0 is about ten times lower than at pH = 7.0. Competition with other cations, in particular with Ca^{2+} , also inhibits Cd sorption (Christensen, 1984; Alloway, 1995).

Although mostly sorbed on the solid phase, its physico-chemical characteristics make Cd more mobile from solid to liquid and therefore more available for root absorption than most other trace metals. When applied to variably contaminated surface horizons, isotopic dilution showed that on average, 61% (9% to 92%) of Cd was isotopically exchangeable, i.e. in equilibrium with the solution content and likely to dissolve in case of the depletion of the solution (Degryse *et al.*, 2004). In another study, it was shown that 46% of Cd in cultivated soils was isotopically exchangeable (Sterckeman *et al.*, 2009).

As a consequence of its chemistry, Cd availability for absorption by roots increases with the soil Cd content and acidity, as well as with the Ca^{2+} in soil solution, and decreases when soil sorption capacity, in particular soil organic matter, increases (Lin *et al.*, 2016b).

III. Cadmium species absorbed by the roots

Most studies suggest or consider that the main absorbed Cd species is Cd^{2+} , i.e. the free ion (Welch and Norvell, 1999). Custos *et al.* (2014) showed that when reducing the concentration of Cd^{2+} in nutrient solution by chelating it by EDTA, the absorption of the metal by maize in hydroponics was strongly decreased. However, Cd complexes (or chelates) can contribute to root uptake in two ways. Firstly, it has been reported that organo-metallic complexes can dissociate in the vicinity of the root surface, as the solution is depleted in free ions due to absorption by the root (Degryse *et al.*, 2006; Panfili *et al.*, 2009; Wang *et al.*, 2009). Therefore, the pool of complexed Cd contributes to the buffering of the free Cd pool.

258 However, this contribution was shown to be very small. Through modelling, Lin *et al.*
 259 (2016b) estimated that in most cases, the dissociation of Cd complexes is slower than
 260 desorption from the solid phase and not fast enough to supply the roots with Cd^{2+} .

261 The other contribution of the complexed Cd would be through direct absorption. However,
 262 this seems variable according to the experimental conditions and has never been directly
 263 demonstrated. According to Panfili *et al.* (2009), durum wheat (*Triticum turgidum* L. ssp.
 264 *durum*) is not able to absorb Cd-citrate complexes, in opposition to what Berkelaar and Hale
 265 (2003a, 2003b) suggested. However, different experiments have shown the presence of Cd-,
 266 Pb- and Zn-EDTA complexes in plant tissues, suggesting that they may be absorbed by roots
 267 (Sarret *et al.*, 2001; Collins *et al.*, 2002; Schaider *et al.*, 2006). The uptake of Cd by maize in
 268 hydroponics being greater than that predicted by a model based on the sole absorption of
 269 Cd^{2+} , Custos *et al.* (2014) also suggested that Cd-EDTA was absorbed from the nutrient
 270 solution. Blaylock *et al.* (1997) showed that an addition of EDTA and other
 271 aminopolycarboxylates like EGTA or DTPA to soil, increases the accumulation of Cd in
 272 Indian mustard, although it is not excluded that this could be the consequence of the
 273 damaging of the endodermis by the chelates (Nowack *et al.*, 2006). Cadmium complexes
 274 would enter the plant through the apoplast at sites lacking apoplastic barriers, i.e. at the root
 275 apex (Redjala *et al.*, 2011) and at the emergence of lateral roots. The driver of the complex
 276 uptake would be the water flow caused by the plant transpiration (Nowack *et al.*, 2006; Custos
 277 *et al.*, 2014), although it is not clear how the complexes would cross cell membranes.

278 Some studies have shown a positive effect of chloride on Cd uptake, in both soil and
 279 hydroponics (Smolders and McLaughlin, 1996a, 1996b; McLaughlin *et al.*, 1997; Weggler *et al.*,
 280 2004). Two mechanisms could account for this effect: (i) the formation of CdCl_n^{2-n}
 281 complexes could favour the desorption of the metal from the soil solid phase, (ii) the
 282 complexes (CdCl_n^{2-n}) could diffuse faster than the free ion in the rhizosphere or in the

apoplast. Absorption of the complexes themselves is not excluded, as CdCl^+ could cross the plasma membrane through selective ion channels for monovalent cations such as K^+ . In addition, the neutral form CdCl_2^0 could passively cross the lipid bilayer (Welch and Norvell, 1999).

Analogous effects, but less intense than with Cl^- , have been obtained with SO_4^{2-} . The Cd concentration in the soil solution increases upon addition of sulphate, which slightly increases Cd absorption (McLaughlin *et al.*, 1998b). An experiment in hydroponics suggests that the CdSO_4^0 complex is taken up by plants as efficiently as free Cd^{2+} ion (McLaughlin *et al.*, 1998a). The electrically neutral CdSO_4^0 complex would passively diffuse across the plasma membrane.

IV. Influence of the plant on soil Cd availability

In theory, roots can influence the concentration and speciation of Cd in the rhizosphere and consequently their ability to take up the metal. Indeed, according to the charge balance resulting from the absorption of the nutritive ions, the roots compensate the imbalance by excreting protons (H^+) or hydroxyls (OH^-) (Hinsinger *et al.*, 2003). In the first case, the rhizosphere acidification would favour the desorption of Cd^{2+} from the soil solid phase while in the second, the alkalization would increase the sorption of the metal (Eq. 8). Acidification of the rhizosphere should therefore increase root Cd uptake, in contrast to alkalization.

However, this effect could only rarely be demonstrated in the case of Cd. Wu (1989) found that nitrogen fertilization of ryegrass using NH_4^+ increased plant Cd uptake, compared with fertilization using nitrate. He attributed this observation to the increase in soil acidity following the higher proton release by the roots absorbing ammonium.

The excretion of organic ligands such as organic acids (Jones *et al.*, 2009) or phytosiderophores could also cause changes in Cd availability in the rhizosphere. Using

isotopic dilution, Sterckeman *et al.* (2005) found an increase in the availability of Cd in the rhizosphere (measured by *L* value) compared to bulk soil (represented by the *E* value). This was the case in an alkaline soil but not in a slightly acidic one. In the alkaline soil, the pool of isotopically exchangeable Cd (i.e. Cd in solution and on the solid phase in equilibrium with that in solution) was greater in the rhizosphere than that in the bulk soil. Some reactions, due to changes in the chemical composition of the rhizosphere soil might therefore have modified the Cd speciation. However, these reactions as well as their origin (roots or associated microflora) are unknown. The increase in Cd availability varied according to the six plant species tested. It was maximal for ryegrass and nil for the hyperaccumulator *N. caerulescens*, in agreement with the results reported by Hutchinson *et al.* (2000) and Zhao *et al.* (2001) who also showed that root exudates of *N. caerulescens* did not increase desorption of Cd and Zn.

Organic acids can increase the solubility of soil Cd, displacing ions adsorbed on the solid phase into the solution as they complex the dissolved free ions (Naidu and Harter, 1998). Whether they favour Cd uptake (Cieslinski *et al.*, 1998) or decrease it through the formation of Cd complexes which are not absorbable by the roots (Pinto *et al.*, 2008) is a matter of debate (as discussed [above](#)). Oxalate excreted by tomato roots in response to Cd exposure has been shown to reduce the metal toxicity, probably because Cd oxalate complexes are excluded from the roots (Zhu *et al.*, 2011). In contrast, Tao *et al.* (2015) suggested that the strong excretion of oxalate at the root apex of the Cd hyperaccumulating accession of *S. alfredii* may favour Cd uptake and accumulation. The mechanisms underlying this observation are however, not clearly identified. They could involve the dissociation of the complex at the root surface, a direct uptake of the chelate through specific transport systems or an apoplastic absorption of the chelates.

Phytosiderophores, such as mugineic and deoxy mugineic acids (MA, DMA), are iron-complexing agents synthesized from nicotianamine and secreted by grasses suffering from a

332 Fe deficiency (Shojima *et al.*, 1990). Experiments with rice (*Oryza sativa*) and maize suggest
 333 that phytosiderophores may favour the availability and uptake of Zn (Zhang *et al.*, 1989; von
 334 Wirén *et al.*, 1996; Arnold *et al.*, 2010; Ptashnyk *et al.*, 2011). However, their effect on Cd
 335 uptake has not been completely clarified. Puschenreiter *et al.* (2017) observed enhanced Zn,
 336 Cu, Mn and Ni concentrations in wheat shoots on some soils associated with
 337 phytosiderophore exudation in Fe-deficient wheat (*Triticum aestivum*). However, plant Cd
 338 concentration did not increase. Accordingly, an addition of DMA to a soil suspension did not
 339 increase the Cd concentration in the solution. In contrast, the work of Shenker *et al.* (2001)
 340 indicated that phytosiderophores are able to increase Cd desorption from the soil solid phase.
 341 However, as in the work of Puschenreiter *et al.* (2017), they did not cause an increase in metal
 342 uptake by wheat or barley (*Hordeum vulgare*). With maize cultivated in hydroponics, Hill *et*
 343 *al.* (2002) showed that Cd stress, as with Fe deficiency, causes greater DMA production by
 344 roots, while DMA appears to limit Cd accumulation. These results suggest that
 345 phytosiderophores can form complexes with Cd which may contribute to the desorption of Cd
 346 from the solid phase of the soil, but prevent its internalization by roots. Meda *et al.* (2007)
 347 partly contradicted this model, showing that Cd-induced phytosiderophore release cannot
 348 protect maize plants from Cd toxicity. Instead, phytosiderophore-mediated Fe acquisition
 349 would provide an advantage under Cd stress by improving Fe uptake. Accordingly, Astolfi *et*
 350 *al.* (2014) showed that most of the genes involved in phytosiderophore biosynthesis and
 351 secretion induced by Fe deprivation were also significantly upregulated in the presence of Cd
 352 under Fe sufficient conditions in barley. Interestingly, the enhanced expression of
 353 phytosiderophore biosynthesis genes in roots was associated with the downregulation of Cd
 354 uptake transporter genes (see [below](#)). The downregulation of Cd uptake transporter expression
 355 would constitute a protective mechanism against further Cd uptake by these transporters.

V. Absorption of Cd by the roots

A. Location of Cd uptake on the root

Laporte *et al.* (2013) showed that in sunflower first order lateral roots, Cd²⁺ influx was 2.9 times higher at root tip than at the root base. These results are consistent with those of Pineros *et al.* (1998), who observed significantly higher Cd influx at positions 1 to 1.5 mm from the root apex of wheat and *N. caerulea* seedlings. Similar profiles were observed in various young roots, in durum wheat (*T. turgidum* L. ssp. *durum*) (Farrell *et al.*, 2005), in poplar (*Populus* × *canescens* plantlets) (He *et al.*, 2011), in rice (Chen *et al.*, 2018b) and in the halophyte *Suaeda salsa* (Li *et al.*, 2012a). These profiles can be attributed to a higher activity of the transport systems close to the root tip, where they could be expressed at higher levels (Chen *et al.*, 2018b) and/or benefit from more energy (Laporte *et al.*, 2013). Higher Cd influx at the root apex could also result from the incomplete development of apoplastic barriers, thereby favouring apoplastic Cd uptake (Redjala *et al.*, 2011) in this part of the root. The Casparian strips and suberin lamellae in the endodermis and exodermis are formed away from the root apex in more differentiated parts of the roots. However, according to the modelling carried out by Laporte *et al.* (2013), the higher influx at the root tip would not significantly influence the overall Cd root uptake, whereas the total root surface area is indeed a major parameter.

B. Mechanisms of Cd root absorption

There is little evidence of a biological role for cadmium. Its involvement in the enzymatic system of a diatom was mentioned above (Lane *et al.*, 2005). As in the case of Zn and Ni, Cd accumulation has been proposed as a defence against herbivory of hyperaccumulating plants (Jiang *et al.*, 2005; Plaza *et al.*, 2015). It is therefore very unlikely that transport systems with specificity for a non-essential and even highly toxic metal were selected during evolution. Instead, Cd is probably taken up through the plasma membrane of root cells by transporters

for essential elements with insufficient selectivity with respect to their substrate (Clemens, 2001).

Cadmium transport into roots was first studied through absorption kinetics, formalized using the Michaelis-Menten model (Cataldo *et al.*, 1983; Costa and Morel, 1994; Cohen *et al.*, 1998; Hart *et al.*, 1998; Redjala *et al.*, 2010a). Although considered as not Cd specific, the transport systems involved show a high affinity to the metal, with K_m falling in the range of 0.02 to 1 μM . For instance, the K_m values for Cd were 0.059 μM and 0.067 μM in bread wheat and durum wheat, respectively, whereas for Zn, a micro-nutrient, they were 2.3 μM and 3.3 μM (Hart *et al.*, 2002). The maximum influx (V_{max}) values ranged from 12 to 188 $\text{nmol (gFW)}^{-1} \text{ h}^{-1}$ (Lux *et al.*, 2011). The highest values were found for the Ganges populations of the metal hyperaccumulator *N. caerulescens* under Fe deficient conditions (Lombi *et al.*, 2002). Cohen *et al.* (1998) also found that Fe deficiency stimulated high affinity Cd uptake in *Pisum sativum* roots. Lombi *et al.* (2001) measured the K_m and V_{max} of the Prayon (low Cd accumulator) and Ganges (high Cd accumulators) populations of *N. caerulescens*. They found identical K_m values (around 0.2 μM), but the V_{max} of the Ganges population (160 $\text{nmol (gFW)}^{-1} \text{ h}^{-1}$) was significantly higher than that of the Prayon population (33 $\text{nmol (gFW)}^{-1} \text{ h}^{-1}$). Similar V_{max} differences were found in the comparison between Calamine (Viviez) and Serpentine (Vosges) populations of the same species (Redjala *et al.*, 2009). Since the V_{max} value is proportional to the density of membrane transporters, the difference in Cd uptake ability by the Prayon and Ganges populations could be due to a difference in the expression of a certain transporter gene with high Cd affinity.

Absorption kinetics are often biphasic in appearance, resulting from the combination of Michaelis-Menten kinetics and linear kinetics. The linear component has often been attributed to adsorption of Cd^{2+} on the apoplasm and therefore to incomplete desorption before the determination of Cd in the root (Hart *et al.*, 1998; Lombi *et al.*, 2001). However, this

interpretation was invalidated using Cd measurement in the symplasm and apoplasm [Redjala (2009); Redjala *et al.* (2010a)]. The linear component would rather correspond to the entry of Cd into the symplasm *via* low affinity transport systems, such as ion channels for Ca^{2+} (Perfus-Barbeoch *et al.*, 2002; Rodriguez-Hernandez *et al.*, 2015; Chen *et al.*, 2018b). In any case, this linear component significantly contributes to the root uptake only at high concentrations of Cd^{2+} in the medium, which do not occur in agricultural soil and are rare in contaminated soils.

In mouse-ear cress (*Arabidopsis thaliana*) (Figure 2, Table 1), Eide *et al.* (1996) showed that Fe deficiency induces the expression of an iron regulated transporter IRT1 (from the zinc-regulated transporter, iron-regulated transporter protein - ZIP family), which allows the transport of divalent metals such as Cd^{2+} , Co^{2+} , Mn^{2+} and Zn^{2+} , in addition to Fe^{2+} , when expressed in yeast (Eide *et al.*, 1996; Korshunova *et al.*, 1999). AtIRT1 is mainly expressed in root epidermis and to a lesser extent in root cortex (Vert *et al.*, 2002). Cd accumulation is strongly reduced in an *irt1* knockout mutant under Fe deficient conditions (Vert *et al.*, 2002). Cohen *et al.* (1998) proposed that the up-regulation of an IRT1 orthologue accounts for elevated Cd uptake under Fe deficiency in *Pisum sativum*. Even though the IRT1 homologue in barley is also able to transport Fe^{2+} , Zn^{2+} , and Cd^{2+} when expressed in yeast (Pedas *et al.* (2008); (Wu *et al.*, 2016a), *in planta*, HvIRT1 mainly controls Mn^{2+} uptake (Long *et al.*, 2018). In rice (Figure 3, Table 1), OsIRT1, as well as its homologue OsIRT2, are predominantly expressed in roots in Fe deficient conditions and are able to transport Cd^{2+} when expressed in yeast. Iron deficiency both enhanced their expression as well as Cd uptake and translocation (Ishimaru *et al.*, 2006; Nakanishi *et al.*, 2006). IRT1 may also be involved in Cd uptake by the hyperaccumulator *N. caerulescens* (Lombi *et al.*, 2002; Plaza *et al.*, 2007), but whether its activity accounts for the particularly high ability of the Ganges population to accumulate Cd remains to be formally demonstrated. In this species (Figure 4),

431 NcZNT1, the orthologue of AtZIP4, was presented as mediating high-affinity Zn^{2+} uptake as
 432 well as low affinity Cd^{2+} uptake (Pence *et al.*, 2000)(Table1). However, this finding was
 433 contradicted by Milner *et al.* (2012) who reported that NcZNT1 can transport Zn, but not Cd
 434 when expressed in yeast or *A. thaliana*. More recently, the results of Lin *et al.* (2016a)
 435 confirmed those of Pence *et al.* (2000). They explained the discrepancy by the fact that the
 436 NcZNT1 cDNA sequence used by Milner *et al.* (2012) lacked the original ATG start codon,
 437 which would have affected the normal function and cellular localization of the aberrant
 438 NcZNT1 protein.

439 Transport proteins of the NRAMP (natural resistance-associated macrophage protein) family
 440 are also involved in the Cd uptake and more generally in its transport in plants. AtNRAMP
 441 1/3/4 transport Fe^{2+} , Mn^{2+} and Cd^{2+} when expressed in yeast (Thomine *et al.*, 2000).
 442 AtNRAMP3/4 are involved in Fe, Mn and Cd efflux from the vacuole (Lanquar *et al.*, 2005;
 443 Lanquar *et al.*, 2010; Pottier *et al.*, 2015). AtNRAMP1 is responsible for high-affinity Mn
 444 uptake by *A. thaliana* roots (Cailliatte *et al.*, 2010). Its role in Cd uptake *in planta* has yet to
 445 be investigated. In rice, OsNRAMP1, which transports Fe, was suggested to contribute to Cd
 446 uptake and transport within rice plants, but did not transport Mn (Takahashi *et al.*, 2011)
 447 (Table 1), while NRAMP1 from *Malus baccata* may be involved in Mn, as well as for Fe and
 448 Cd transport (Xiao *et al.*, 2008). OsNRAMP5 turned out to be the major transporter for the
 449 uptake of Mn and Cd in rice roots (Ishikawa *et al.*, 2012; Ishimaru *et al.*, 2012; Sasaki *et al.*,
 450 2012; Tang *et al.*, 2017) (Table 1). Ishimaru *et al.* (2012) reported that OsNRAMP5 is
 451 expressed in the epidermis, exodermis, and outer layers of the cortex as well as in tissues
 452 around the xylem. Sasaki *et al.* (2012) detected the protein only in exodermis and endodermis.
 453 OsNRAMP5 loss of function mutants are defective when it comes to high affinity Cd uptake
 454 into the roots (Sasaki *et al.*, 2012) and in Cd translocation from roots to shoots (Yang *et al.*,
 455 2014). Consequently, these mutants accumulate Cd at extremely low levels in all organs

including grain. In *Triticum polonicum*, TPNRAMP5 is mainly expressed in roots and basal stems, and the protein is localized in the plasma membrane. Expression of TPNRAMP5 in *A. thaliana* enhanced the accumulation of Cd, Co and Mn, but not that of Zn and Fe (Peng *et al.*, 2018). The NRAMP5 transporter is also present in maize and in bread wheat, but displays a lower level of expression and a lower activity than in rice (Sui *et al.*, 2018).

Several root metal uptake transporters display a polar localization. OsNRAMP5 and AtIRT1, that have been shown to mediate Cd uptake are concentrated on the membrane facing the soil solution (Sasaki *et al.*, 2012; Barberon *et al.*, 2014). This raises the question of how metals are transferred towards the stele, from the other side of the cell facing the central cylinder. In plants, members of the cation diffusion facilitator (CDF) family are known as metal tolerance proteins (MTP). They are involved in metal efflux from the cytosol to other compartments: the apoplast, the vacuole and the Trans Golgi Network (TGN). In rice, Ueno *et al.* (2015) showed that OsMTP9 is polarized on the side of the plasma membrane facing the stele and is required for efficient Mn uptake (Table 1). Working on cucumber (*Cucumis sativus*), Migocka *et al.* (2015) found that the CsMTP9 gene encodes a plasma membrane H⁺-coupled Mn²⁺ and a Cd²⁺ antiporter expressed exclusively in roots. CsMTP9 was shown to be involved in the efflux of Mn and Cd from root cells, thereby allowing the transport of both metals from the endodermis into the vascular cylinder for further translocation to the shoot.

Some authors have suggested that Cd could also enter cells through Ca²⁺ channels (Welch and Norvell, 1999; Perfus-Barbeoch *et al.*, 2002; Li *et al.*, 2012b). This is supported both by the finding that Cd influx is affected by Ca²⁺ channels blockers (Cohen *et al.*, 1998), as well as by the correlation between Cd and Ca accumulation in numerous non-metallicolous populations of *N. caerulescens* grown (Sterckeman *et al.*, 2017). The work of Chen *et al.* (2018b) on rice indicates that the Ca channels OsANN4 and OsGLR3.4 can mediate Cd uptake in roots (Table

1). However, their results were obtained after exposing the roots to 20 μM Cd, which is about 1000 times higher than the concentration plants are exposed to in field conditions.

The existence of a root efflux system for Cd was suggested quite a long time ago, in particular by Costa and Morel (1993) from the absorption kinetics of white lupin (*Lupinus albus*). This hypothesis was further reinforced by studies showing that a small portion of the radioactive Cd deposited on lettuce, radish and bean leaves was transferred to the rhizosphere soil (Fismes *et al.*, 2005). More recently, the PDR8 protein (pleiotropic drug resistance), expressed in all cells of *A. thaliana*, but more intensely in the root hairs and epidermis, has been shown to be responsible for Cd efflux (Kim *et al.*, 2007) (Table 1). This protein belongs to the large family of ATP-binding cassette (ABC) transporters, known for transporting a broad range of substrates, including ions, carbohydrates, lipids, xenobiotics, drugs and toxic metals. In addition, a small Cys-rich membrane protein, AtPCR1 (Arabidopsis plant cadmium resistance), is also able to reduce Cd accumulation when expressed either in yeast or *A. thaliana* protoplasts (Song *et al.*, 2004). This protein is localized in the plasma membrane and expressed in both roots and shoot. In contrast to AtPCR2, which is a Zn efflux transporter (Song *et al.*, 2010), AtPCR1 does not seem to act as an efflux transporter and the mechanism by which it confers Cd resistance to the plant remains unclear.

VI. Complexation of Cd by organic ligands in the plant

Cadmium can form complexes with organic ligands both in the cytosol and the vacuole. This reaction is generally considered to be a detoxification process occurring in the roots as well as in the shoots (Vögeli-Lange and Wagner, 1990; Cobbett and Goldsbrough, 2002; Clemens, 2006; Verbruggen *et al.*, 2009a). In the articles reporting research into this process, it is often not easy to distinguish the plant organs in which it takes place. This is the reason why, unless indicated, this part of the review considers Cd complexation in both roots and shoots.

504 A. *Glutathione and phytochelatins*

505 Glutathione (GSH) and phytochelatins (PCs), are non-proteinogenic peptides involved in Cd
506 detoxification. PCs have been extensively studied since they were discovered (Grill *et al.*,
507 1985). In roots of non-hypertolerant plants, most Cd is chelated by PCs (Verbruggen *et al.*,
508 2009a), yet GSH and PCs also bind Cd in the shoot.

509 GSH (Glu-Cys-Gly), which is essential for the cellular redox balance can form complexes
510 with various metals. Upon metal or metalloid excess, PCs, with the structure: (γ -Glu-
511 Cys)_nGly, where n = 2-11) are synthesized from GSH by the phytochelatin synthase (PCS)
512 (Grill *et al.*, 1989; Clemens *et al.*, 1999; Ha *et al.*, 1999; Vatamaniuk *et al.*, 1999; Vatamaniuk
513 *et al.*, 2000). The gene encoding PCS was shown to be essential for Cd and As tolerance (Ha
514 *et al.*, 1999). In numerous plant species, the synthesis of PCs is induced by exposure to Cd
515 (Salt *et al.*, 1997; Schat *et al.*, 2002; Hernandez-Allica *et al.*, 2006; Vazquez *et al.*, 2006;
516 Lindberg *et al.*, 2007). The relatively soft acid character of Cd²⁺ leads to a strong affinity for
517 soft sulphur donor atoms, especially for thiolates, for instance in cysteine residues (Mah and
518 Jalilehvand, 2010). The Cd-GSH and Cd-PC complexes have been determined and
519 characterized *in vitro* (Cruz *et al.*, 2005; Chekmeneva *et al.*, 2007; Leverrier *et al.*, 2007;
520 Chekmeneva *et al.*, 2008; Mah and Jalilehvand, 2010; Jacquart *et al.*, 2017). *In vivo*, the
521 existence, the distribution and the formation kinetics of Cd-PC complexes was supported by
522 analyses using X-ray absorption spectroscopy (Salt *et al.*, 1995; Salt *et al.*, 1997), small-scale
523 gel filtration (Rauser, 2003) or fractionation techniques coupled to mass spectrometry
524 (Persson *et al.*, 2006).

525 The first step of the detoxification process is the binding of Cd²⁺ with GSH to form
526 bisglutathionate-Cd complex, GS₂-Cd(II). GS₂-Cd(II) interacts with the phytochelatin
527 synthase, a constitutively expressed enzyme, which triggers the phytochelatin synthesis and
528 the formation of PC-Cd(II) low molecular weight complexes (LMW PC-Cd) (Vatamaniuk *et*

529 *al.*, 2000). LMW PC-Cd complexes are then transported into the vacuole *via* an ABC-type
 530 transporter (see [below](#)) to form high molecular weight, sulphide-containing complexes (HMW
 531 PC-Cd S)(Rauser, 1995).

532 Phytochelatin production can increase plant tolerance to Cd (Persson *et al.*, 2006; Gasic and
 533 Korban, 2007; Brunetti *et al.*, 2015), but this cannot be generalized. Comparing a Cd
 534 accumulating and tolerant cultivar of black oat (*Avena sativa*) to a Cd-sensitive one, Uraguchi
 535 *et al.* (2009a) observed that Cd exposure increased PC concentrations in both cultivars, thus
 536 indicating that the differences between the cultivars could not be accounted for by difference
 537 in PC synthesis. In this case, increased storage in the cell wall would appear to account for
 538 enhanced tolerance and accumulation of Cd ([see below](#)). Overexpression of AtPCS1 in
 539 Arabidopsis was even found in some instances to increase Cd sensitivity (Lee *et al.*, 2003).
 540 This was attributed to a detrimental depletion of GSH in the overexpression lines.

541 The role of PCs in adaptation to Cd-contaminated soils has been questioned by the work of
 542 Leopold *et al.* (1999), who showed that Cd-complexes disappeared after 7 to 14 days of Cd
 543 exposure in the metal tolerant *Silene vulgaris*. This result indicated that synthesis of metal-
 544 PC-complexes was transient and not necessarily important for long-term metal tolerance by
 545 plants. Wójcik *et al.* (2006) also observed that PC concentration increases with the increasing
 546 Cd concentration to which *N. caerulea* is exposed, but decreases with exposure time. PCs
 547 seem to play a minor role in Cd tolerance by this hyperaccumulator (Wojcik *et al.*, 2005b;
 548 Wójcik *et al.*, 2006). This is consistent with the fact that in young leaves of *N. caerulea*,
 549 around 25% of the Cd ligands were S ligands (e.g. phytochelatins, and other Cys-rich
 550 peptides), and the proportion of S ligands decreased with increasing leaf age. In senescent
 551 leaves, almost exclusively O ligands were found (Küpper *et al.*, 2004). Schat *et al.* (2002);
 552 Wójcik *et al.* (2006) suggested that adaptive Cd tolerance was not dependent on PC-mediated
 553 sequestration. For Sun *et al.* (2007a), PCs are not involved in Cd transport,

hyperaccumulation or tolerance in the metal-hypertolerant population of *S. alfredii*, while Zhang *et al.* (2010) showed that PCs do not detoxify Cd in the roots of this species, contrarily to what might occur in the leaves. Comparing different species and organs, Marentes and Rauser (2007) showed that phytochelatins were not necessarily the major Cd ligands. Sun *et al.* (2007b) concluded that PC synthesis was not the primary reason for Cd tolerance in *Solanum nigrum*. Moreover, strikingly, genes involved in phytochelatin synthesis or transport have not been identified in transcriptomic or genetic analyses of Cd hyperaccumulation or hypertolerance.

In addition, PCs have been implicated in long distance Cd transport. Expressing wheat PCS specifically in *A. thaliana* roots, Gong *et al.* (2003) found that PCs synthesized in roots are transported to the shoots and that this correlates with enhanced Cd transfer from root to shoot. However, later studies indicated that this is not the main pathway for Cd root to shoot transfer (Wong and Cobbett, 2009). Using grafts between a phytochelatin-deficient Arabidopsis mutant and the wild type, Chen *et al.* (2006) demonstrated that phytochelatins are also transported from shoot to roots.

B. Metallothioneins and other cysteine-rich polypeptides

Metallothioneins (MTs) are cysteine rich, low molecular weight, gene-encoded, metal-binding polypeptides that are ubiquitously found in eukaryotes and cyanobacteria (Cobbett and Goldsbrough, 2002). It has been shown that MTs play an important role in metal homeostasis and tolerance in plants (Zimeri *et al.*, 2005; Guo *et al.*, 2008; Ren *et al.*, 2012; Benatti *et al.*, 2014). Elevated expression of MT genes (MT1, MT2, MT3) has frequently been observed in metal-hyperaccumulating and hypertolerant species or populations (van Hoof *et al.*, 2001; Mengoni *et al.*, 2003; Roosens *et al.*, 2005; Chiang *et al.*, 2006; van de Mortel *et al.*, 2006; Hassinen *et al.*, 2007; Jack *et al.*, 2007). However, no positive correlation between these expression levels and metal tolerance/accumulation was observed. Recently, Peng *et al.*

(2017a) proposed that the cysteine-rich MT-like protein SpMTL could act as a cytosolic chelator to detoxify Cd in the hyperaccumulator *S. plumbizincicola* (Table 1). However, this role has to be confirmed in other species.

In *Digitaria ciliaris*, Kuramata *et al.* (2009) identified a novel gene, DcCDT1 (*D. ciliaris* cadmium tolerance), encoding a 55 amino acid peptide, containing 15 (27%) cysteine residues, as well as its rice homolog, OsCDT1 (Table 1). When constitutively expressed in yeast cells and *A. thaliana*, this gene enhanced their Cd tolerance by limiting cellular Cd accumulation. The authors proposed that the peptide is secreted outside the cell, where it partly associates with the cell wall. It then prevents Cd internalization by chelating the metal at the cellular surface.

C. Organic acids

Carboxylic acids also contribute to Cd complexation. Salt *et al.* (1997) showed that during the initial stages of Cd uptake (6-12 h) by *Brassica juncea* seedlings, most of the intracellular Cd was bound to oxygen atoms, possibly from organic acids. The spectra of pure Cd citrate fitted relatively well with the spectra of *B. juncea* seedlings. Later, Ueno *et al.* (2005) showed that Cd is mainly associated to malate in the leaves of *N. caerulea*. In roots and shoots of the hyperaccumulator *Thlaspi praecox*, Vogel-Mikuš *et al.* (2010) found that up to 80% of the Cd ligands are oxygen ligands provided by the cell walls (see below) and by organic acids stored in the vacuoles. In the stems and leaves of *Gomphrena claussenii*, a Cd tolerant species, Cd was mainly bound to oxalate in crystals also containing Ca. The plant was however, exposed to very high Cd concentrations in hydroponics (100 μ M CdSO₄). In the roots, there was no oxalate crystals and Cd was predominantly associated to S ligands (Pongrac *et al.*, 2018). The complexation of Cd by organic acids is particularly important in the epidermal cells, where the vacuoles represent 99% of the symplast volume. In the stems and leaves of the Cd hyperaccumulating ecotype of *S. alfredii*, Cd is mainly associated with oxygen ligands and the

majority (about 70% in leaves and 47% in stems) is bound to malic acid, i.e. the major organic acid in the shoots (Tian *et al.*, 2011).

VII. Storage of Cd in vacuoles of roots and shoots

The vacuole is an important storage organelle for many ions. As with complexation with organic ligands, transport from the cytosol into the vacuole is regarded as a process increasing plant tolerance and plant ability to accumulate high levels of toxic metallic ions, occurring in either the roots or the shoots. In the leaves of *N. caerulea*, Wojcik *et al.* (2005a) found that Cd accumulates in vacuoles and suggested these are the main compartments for storage and detoxification of this metal. In the literature, it is sometimes difficult to distinguish between root and aerial parts with respect to the nature of the Cd transporters present in the vacuole membrane, the tonoplast. This is why this part of the review concerns storage in vacuoles in both below- and above-ground organs.

Several transport pathways mediate Cd sequestration in the vacuole either as free Cd or PC-Cd complexes. They use either ATP hydrolysis or the proton gradient across the vacuolar membrane as sources of energy to drive substrate accumulation inside the vacuole. Cadmium can be transported into the vacuole by means of a H⁺/cation-antiporters designated as CAX (CAtion eXchanger), that use the proton gradient to mediate divalent cation storage in the vacuole (Salt and Wagner, 1993; Hirschi *et al.*, 2000; Pittman *et al.*, 2004; Shigaki and Hirschi, 2006; Shen *et al.*, 2012). Among the members of the CAX family that have been characterized as calcium transporters, CAX1, CAX2 and CAX4 have the ability to transport Cd as well (Table 1). In *A. thaliana*, CAX2 is strongly expressed in the leaf vascular tissue, in vegetative meristematic tissue, as well as in the root vascular tissue and in the root tip (Pittman *et al.*, 2004). Koren'kov *et al.* (2007) compared seven different CAX genes from *A.*

628 *thaliana* in transformed tobacco. They showed that all the genes are involved in the transport
 629 of Cd^{2+} , Ca^{2+} , Zn^{2+} , and Mn^{2+} to varying degrees, but that AtCAX4 and AtCAX2 have a
 630 higher Cd^{2+} selectivity. AtCAX4 is expressed at higher levels in the roots. Korenkov *et al.*
 631 (2009) showed that expression of AtCAX2 or AtCAX4 in transformed tobacco roots reduces
 632 Cd translocation to the aerial parts by promoting sequestration of the metal in the roots. In
 633 addition, CAX1a is expressed in vacuoles of barley (*H. vulgare*) exposed to Cd, thus
 634 suggesting that this protein plays a role in Cd vacuolar storage (Schneider *et al.*, 2009).
 635 Expression of AtCAX1 in petunia enhanced Cd tolerance and accumulation (Wu *et al.*, 2011).
 636 Moreover, Baliardini *et al.* (2015) suggested that the role of CAX1 in Cd tolerance is not
 637 linked to Cd transport but in maintaining cytosolic Ca levels to avoid uncontrolled ROS
 638 accumulation. The evidence that CAX transporters are involved in Cd transport *in planta*
 639 come from overexpression experiments, whereas loss of function would be necessary to
 640 determine their actual contribution to Cd storage and Cd tolerance. Membrane transporters of
 641 the CDF family do not seem to be involved into vacuolar Cd sequestration. Indeed, neither
 642 vacuolar MTP from *A. thaliana* nor its hyperaccumulator relative *A. halleri* displayed Cd
 643 transport activity (Dräger *et al.*, 2004; Desbrosses-Fonrouge *et al.*, 2005; Arrivault *et al.*,
 644 2006). Accordingly, MTP1 was identified as a QTL for Zn tolerance, but not for Cd tolerance
 645 in *A. halleri* (Courbot *et al.*, 2007; Willems *et al.*, 2007).

646 The P_{1B}-ATPase subfamily HMA (for Heavy Metal Associated) also plays a role in Cd
 647 sequestration in vacuoles. Morel *et al.* (2009) showed that AtHMA3 is located in the vacuolar
 648 membrane of *A. thaliana* (Table 1). In shoots, AtHMA3 has a high expression level in guard
 649 cells, hydathodes, vascular tissues, while in roots, it is expressed in the stellar cells and at the
 650 apex. AtHMA3 participates in the vacuolar storage of Cd, improving plant tolerance to the
 651 metal (Morel *et al.*, 2009). A homologous gene, OsHMA3, is a major determinant of the
 652 variation in Cd accumulation in shoots and grains among rice cultivars (*O. sativa*) (Ueno *et*

653 *al.*, 2010; Miyadate *et al.*, 2011; Sui *et al.*, 2019). OsHMA3 is mainly expressed in the
 654 tonoplast of root cells. OsHMA3 was found to limit Cd translocation from roots to shoot by
 655 selectively sequestering Cd into root vacuoles (Ueno *et al.*, 2010). Mutations leading to a loss
 656 of functionality of the OsHMA3 transporter in the roots explain the elevated accumulation of
 657 Cd in the aerial parts of several indica rice cultivars, Jarjan (Ueno *et al.*, 2011a; Yan *et al.*,
 658 2016), Hitomebore (Tanaka *et al.*, 2016) and Cho-Ko-Koku (Miyadate *et al.*, 2011),
 659 compared to japonica cultivars. Sun *et al.* (2019) showed that a Gly to Arg substitution at
 660 position 512 in OsHMA3 causes some *japonica* rice cultivars to accumulate larger amounts of
 661 Cd in the shoots and grains. Interestingly, variations in the coding sequence of AtHMA3 also
 662 account for the variations in shoot Cd accumulation among *A. thaliana* accessions (Chao *et*
 663 *al.*, 2012), in *Brassica rapa* (Zhang *et al.*, 2019) and in Durum wheat (Maccaferri *et al.*,
 664 2019). Overexpression of a functional OsHMA3 in a japonica rice cultivar further decreased
 665 Cd accumulation in shoots and grains (Ueno *et al.*, 2010). Shao *et al.* (2018) fused a promotor
 666 of OsHMA2 (highly expressed in the node) to OsHMA3. The consequence was an enhanced
 667 expression of OsHMA3 and a modification in the tissue localization of the transporter, which
 668 was also expressed in the nodes. This resulted in a reduced Cd accumulation in grain due to
 669 the sequestration of Cd in the vacuoles of various tissues. The genome of the metal
 670 hyperaccumulator *N. caerulescens* also encodes one or several HMA3 homologues that are
 671 localized in the vacuolar membrane and allow Cd sequestration (Ueno *et al.*, 2011b). In
 672 contrast with rice and *A. thaliana*, NcHMA3 is also strongly expressed in shoot tissues and is
 673 responsible for Cd sequestration in leaf cell vacuoles (Ueno *et al.*, 2011b; Mishra *et al.*,
 674 2017). The latter suggested that greater expression of NcHMA3 would explain the
 675 hyperaccumulation of Cd by certain populations (e.g. Ganges) of this species (Ueno *et al.*,
 676 2011b). In the hyperaccumulator *S. plumbizincicola* (Crassulaceae), SpHMA3 is also strongly
 677 expressed in shoots and is localized in the tonoplast. It plays an important role in Cd

678 detoxification in young leaves and stems by sequestering the metal in the vacuole (Liu *et al.*,
 679 2017). Working on HMA3 from soybean (*Glycine max*), Wang *et al.* (2018) proposed that
 680 GmHMA3 limits the translocation of Cd from the roots to the stems, by sequestering Cd into
 681 the root endoplasmic reticulum (ER). This is surprising, as Cd toxicity would be expected to
 682 be very high in the ER, probably interfering with disulphide bound formation.

683 It has recently been proposed that the homeodomain-containing protein, OsHB4a was
 684 responsible for Cd sensitivity and accumulation in the leaves and grains of rice plants through
 685 the regulation of other genes, such as OsHMA2 and OsHMA3 (Ding *et al.*, 2018). OsHB4a is
 686 up-regulated upon Cd exposure and favours xylem loading and Cd translocation. Moreover,
 687 miR166 a well-conserved miRNA present in all plants, targets OsHB4a for degradation. The
 688 expression of miR166 decreases upon Cd exposure. Cd exposure thus favours its
 689 accumulation and toxicity in rice by simultaneously up-regulating OsHB4a and down-
 690 regulating its inhibitor, miR166.

691 Based on their up-regulation upon Cd exposure and their localization at the vacuolar
 692 membrane, the MRP3 transporters of the ABC family from mouse-ear cress and barley have
 693 been suggested as driving the influx of PC-Cd complexes into the vacuole (Tommasini *et al.*,
 694 1998; Schneider *et al.*, 2009) (Table 1). A similar function has been demonstrated for the
 695 AtMRP7 transporter (Wojas *et al.*, 2009). In the case of AtMRP7 or AtMRP3, whether Cd is
 696 transported as a complex with PC rather than as a free ion, was not determined. However,
 697 Park *et al.* (2012) showed that transporters of the same subfamily, AtABCC1/AtMRP1 and
 698 AtABCC2, are indeed responsible for the sequestration of phytochelatin-Cd complexes into
 699 the root vacuoles of *A. thaliana* (Table 1). Song *et al.* (2014) demonstrated that a vacuolar
 700 transporter for PC-Cd was also present in barley and proposed that it could be an ABC-type
 701 transporter, such as AtABCC1 or AtABCC2. In addition, AtABCC3 increases vacuolar Cd

through the transport of Cd-phytochelatin complexes. Its activity is regulated by Cd and coordinated with that of AtABCC1 and AtABCC2 (Brunetti *et al.*, 2015).

In contrast with CAX, MTPs, HMA and ABCCs, transporters belonging to the NRAMP family participate in Cd efflux from the vacuole (Table 1). In *A. thaliana*, AtNRAMP3 and AtNRAMP4 both reside on the vacuolar membrane (Thomine *et al.*, 2003; Lanquar *et al.*, 2005) and can transport Cd when expressed in yeast (Thomine *et al.*, 2000). Consistent with its redundant function in Cd efflux from root vacuoles, *nramp3nramp4* double mutant in the WS accession, which harbours a functional HMA3 gene, accumulates greater Cd concentrations in roots than the wild type (Chao *et al.*, 2012; Pottier *et al.*, 2015). Conversely, overexpression of AtNRAMP4 decreases root Cd concentration. In the hyperaccumulator *N. caerulescens*, NcNRAMP3 and NcNRAMP4 have the same protein function as their *A. thaliana* homologues, but are expressed at higher levels (Oomen *et al.*, 2009). The efflux of Cd and Zn from root vacuoles driven by NcNRAMP3 and 4 may be required for the efficient translocation of these metals from roots to shoots in *N. caerulescens*.

VIII. Interactions of Cd with the apoplast

A. Transport through the apoplast

The root apoplast is a compulsory pathway for Cd²⁺ and for Cd complexes before entering the symplast. The solute is considered to move radially in the root apoplast by diffusion and advection, before reaching the plasma membrane and crossing it through transport proteins (see above). Cd needs to use the symplastic route to cross the exodermis and/or the endodermis cell layers, where apoplastic transport is blocked by the apoplastic barriers (Casparian strip, suberine lamellae, secondary thickening). It has however been suggested that Cd could reach the xylem *via* the sole apoplastic pathway in the parts of the root where the apoplastic barriers are lacking or incomplete, namely the root tip, stage I endodermis and sites

of lateral root emergence (White, 2001). This hypothesis is supported by studies showing a greater Cd influx along the few millimetres at the root tip (see above).

The relative contributions of the symplastic and apoplastic pathways to Cd loading in the xylem have yet to be determined. The work of Redjala *et al.* (2009); Redjala *et al.* (2010b) showed that apoplastic adsorption had a minor contribution to root Cd uptake, when compared to symplastic uptake at low concentration exposure, such as that in soil solution. Nevertheless, Feng *et al.* (2018) showed that, under Cd stress, endodermal apoplastic barriers were much thicker in a low Cd accumulating sorghum genotype than in a highly accumulating one. The weaker apoplastic barriers were considered to favour xylem loading via the apoplastic pathway in the highly accumulating genotype. Tao *et al.* (2017) highlighted the significance of the apoplastic pathway in Cd accumulation using a Cd hyperaccumulating ecotype of *S. alfredii*. The contribution of apoplast to Cd xylem loading depended on the Cd exposure level and represented up to 37% of total uptake. The main entry sites for apoplastic uptake were in the root apices and at the base of lateral roots, where suberin lamellae are not well-developed.

In the aerial parts, it is difficult to quantify the contribution of the apoplastic transport of Cd. The cell walls of leaves (see below) contain Cd that may migrate into the leaves towards the epidermis, by advection in the network of cell walls driven by the transpiration flow.

B. Storage in the apoplast

Electronegative sites of cellulose, hemicellulose and pectin chains enable the sorption of cations in the cell walls (Haynes, 1980; Grignon and Sentenac, 1991). Sorption in the apoplast is considered, together with chelation, vacuolar sequestration and translocation, as a means of Cd detoxification (Vazquez *et al.*, 2006; Nocito *et al.*, 2011). However, the fraction of the metal stored in this compartment appears to vary widely. When maize and pea (*Pisum sativum*) were cultivated and exposed to Cd in hydroponics, higher Cd levels were found in

751 the cell-wall fraction of leaves and roots of maize than in those of pea. This correlated with
 752 more severe toxicity symptoms in pea (Lozano-Rodriguez *et al.*, 1997). Based on Energy
 753 Dispersive X-ray micro-analysis in the root cortex of *A. thaliana*, Cd accumulated in the cell
 754 wall together with phosphate whereas, in the central cylinder, Cd was present in the cell wall
 755 as Cd/S granular deposits in the middle lamella of the pericycle (Van Belleghem *et al.*, 2007).
 756 In the other parts of the central cylinder, the Cd/S granular deposits were located near the
 757 plasma membrane and not in the middle lamella. In leaves of oilseed rape (*Brassica napus*),
 758 Cd in cell walls represented only 11% of the metal content (Carrier *et al.*, 2003). In tea
 759 seedlings, most of the Cd accumulated in roots, with about 12% to 30% in the cell walls,
 760 while cell wall represented 43.6–83.4% of the very low Cd content in leaves (Cao *et al.*,
 761 2018a). Cosio *et al.* (2005) showed that 33-35% of Cd in the aerial parts of *N. caerulea* is
 762 in the cell walls. In the case of willow (*Salix viminalis*) exposed to high Cd levels, the main
 763 sink of the metal was the pectin layer of collenchyme cell walls, close to the leaf veins
 764 (Vollenweider *et al.*, 2006). Xiong *et al.* (2009) showed that exogenous NO increases Cd
 765 tolerance in rice plantlets by enhancing pectin and hemicelluloses content in the root cell wall,
 766 which is associated with an increase in Cd accumulation in the root cell walls and a decrease
 767 in Cd concentration the soluble fraction of leave cells. Xu *et al.* (2015) showed that, in *A.*
 768 *thaliana*, Cd itself increases the polysaccharide contents and the binding capacity of root cell
 769 walls. Among oat varieties, the highest Cd-resistance coincided with greatest Cd storage in
 770 the cell walls of aerial parts (Uraguchi *et al.*, 2009a). This was also suggested by the work of
 771 Sterckeman *et al.* (2011), which showed an increase in Cd apoplastic fraction in maize and *N.*
 772 *caerulea* roots after exposure to high Cd concentrations. In the hyperaccumulating species
 773 *A. halleri*, Meyer *et al.* (2015) correlated Cd induced modification in the cell wall with
 774 intraspecific variations in Cd tolerance and accumulation. The apoplast also appeared to be an
 775 important storage compartment for Cd in the Cd hyperaccumulator *S. alfredii* (Zhang *et al.*,

2010). Li *et al.* (2015) showed that more Cd was retained in the root cell wall of the non-hyperaccumulating ecotype (NHE) of *S. alfredii* than in the hyperaccumulating one (HE). Transcriptomic analyses also indicated an important role played by the cell wall, in the Cd hyperaccumulation in *S. plumbizincicola* (Peng *et al.*, 2017b). As in *A. thaliana*, Cd treatment induced an increase in cell-wall polysaccharides (pectins and hemicelluloses) in the NHE of *S. alfredii*, but not in the HE. Consequently, more Cd was available for xylem loading in the HE ecotype.

It should be noted that in several of the above-cited works (Lozano-Rodriguez *et al.*, 1997; Carrier *et al.*, 2003; Cao *et al.*, 2018a), as well as in others (e.g. (Fu *et al.*, 2011; Wang *et al.*, 2015a; Wang *et al.*, 2015b), the method used to determine Cd (or other elements) in cell walls is questionable. Indeed, it is based on a fine grinding of the fresh tissues in a buffer solution to destroy the cells. The homogenate is generally then sieved to collect the cell walls. The filtrate is centrifuged to separate chloroplasts from other organelles and from cytosolic and vacuole solution. This procedure in liquid phase does not exclude the diffusion of Cd from one fraction to another during the tissue grinding and fraction separation. The content measured in each fraction might not therefore, correspond to the distribution of the metal *in vivo*.

IX. Translocation and reallocation of Cd

Cadmium concentration is often higher in the roots than in the shoots (Jarvis *et al.*, 1976; Lozano-Rodriguez *et al.*, 1997; Harris and Taylor, 2013; Cornu *et al.*, 2016), and generally lower in the seeds than in the stem and leaves (Hinesly *et al.*, 1978; Wiggerhauser *et al.*, 2016; Chen *et al.*, 2018a). In some species, such as lettuce (*Lactuca sativa*), spinach (*Spinacia oleracea*), cauliflower (*Brassica oleracea*) and oat, concentrations are higher in the shoot than in the roots when plants are grown on low Cd contaminated soils. However, when the plants are grown on more contaminated soil, the root concentration becomes higher than that of the shoots (John, 1973). In maize, Perriguet *et al.* (2008) observed increasingly greater

concentrations in roots than in shoots and an increase in the Cd root-to-shoot ratio, as that of the Cd concentration in the nutrient solution rose. The ratio sharply decreased after reaching a peak, as if the root retention capacities became overloaded at this point. The sequestration of Cd in the roots up to a certain limit corresponds to the excluder strategy proposed by Baker (1981) for certain species. This author also defined an accumulator strategy, “where metals are concentrated in above-ground plant parts from low or high soil levels”. This is the case for *N. caerulescens*, *S. nigrum* or *S. alfredii* (Yang *et al.*, 2004; Wei *et al.*, 2006; Lovy *et al.*, 2013), which can be regarded as Cd hyperaccumulators as the Cd concentration in their shoots can reach 100 mg Cd (kg DW)⁻¹ (van der Ent *et al.*, 2013).

A. Cd loading into the xylem

HMA transporters play an important role in the loading of Cd in xylem vessels. As ATPases, they can transport the cation against its electrochemical gradient, from the cytosol into the xylem sap. AtHMA4 is expressed at its highest level in tissues surrounding the root vascular vessels in *A. thaliana* (Mills *et al.*, 2003; Verret *et al.*, 2004). Located in the plasma membrane and acting as an efflux pump, HMA4 plays a role in metal loading into the xylem, thereby enhancing the root-to-shoot translocation of Zn and Cd in *A. thaliana* (Verret *et al.*, 2004; Mills *et al.*, 2005). Together with AtHMA4, its paralogue AtHMA2 was shown to be involved in the root-to-shoot transfer of Cd (Wong and Cobbett, 2009; Wong *et al.*, 2009) (Table 1). Moreover HMA4 is also important for Cd translocation in the hyperaccumulator *N. caerulescens* (Papoyan and Kochian, 2004). Accordingly, in *A. halleri*, enhanced expression and a triplication of HMA4 was shown to be responsible for the hypertolerance to Cd (Courbot *et al.*, 2007; Hanikenne *et al.*, 2008). In *N. caerulescens*, the HMA4 gene is also present as multiple tandem copies (Ó Lochlainn *et al.*, 2011) and differences in HMA4 expression correspond to the differences in Cd translocation and accumulation among different populations of *N. caerulescens* (Craciun *et al.*, 2012).

In rice, a single gene, OsHMA2, which is orthologous to AtHMA2 and AtHMA4, encodes an efflux transporter involved in Cd xylem loading. OsHMA2 is constitutively expressed in the vascular bundles of roots at the vegetative stage, and knocking-out this gene results in lower root-to-shoot translocation and grain content of Cd (Nocito *et al.*, 2011; Satoh-Nagasawa *et al.*, 2012; Takahashi *et al.*, 2012; Fontanili *et al.*, 2016). OsHMA2 is also expressed in the phloem tissue of nodes (Yamaji *et al.*, 2013). To account for the role of OsHMA2 in the control of Cd transfer between phloem and xylem in nodes, Yamaji *et al.* (2013) proposed that OsHMA2 could function as an influx transporter for both Cd and Zn. Such reverse transport is extremely unlikely, as the function of HMAs in metal efflux has been conserved across kingdoms and the transport mechanism involves ATP hydrolysis at the cytosolic side of the protein. Based on a Cd/Zn reciprocal competition study, Fontanili *et al.* (2016) suggested that, beside OsHMA2, another transport system which is not affected by the presence of Zn^{2+} , yet to be identified, may mediate root-to-shoot Cd translocation in rice. Recently, Hao *et al.* (2018) suggested that OsCCX2, a putative cation/calcium (Ca) exchanger mainly expressed in xylem region of the nodes, could be an efflux transporter, controlling Cd loading into xylem (Table 1).

Defensins are small cysteine-rich proteins, widely distributed in invertebrates, plants, and mammals and known for their involvement in the ancestral non-specific immune response and antifungal activity. Their role in metal homeostasis was first established in a work reporting that this conferred enhanced Zn tolerance in yeast and plant (Mirouze *et al.*, 2006). More recently, CAL1, a defensin-like protein from rice, was found to bind Cd and to drive its efflux from xylem parenchyma cells to extracellular spaces (the apoplast), thus contributing to Cd loading into the xylem sap (Luo *et al.*, 2018) (Table 1). Therefore, the expression of CAL1 leads to an increased Cd concentration in leaves, through increased translocation by the

xylem. Whether the secretion of OsCAL1–Cd complexes indeed involves transporters or vesicular trafficking, is however still unknown.

B. Transport in the xylem

Once loaded into the root xylem, Cd migrates to the aerial parts with the xylem sap flow, driven by plant transpiration, *i.e.* by the gradient of water potential between the soil and the atmosphere. Numerous works show that Cd concentration in aerial plant parts increases with the concentration in the root exposure solution (see for instance Jarvis *et al.* (1976); Florijn and Van Beusichem (1993a); Lovy *et al.* (2013)). Moreover, various investigations have shown that Cd content in the xylem sap increases with Cd content in the exposure solution (Salt *et al.*, 1995; Wei *et al.*, 2007; Ueno *et al.*, 2008; Nakamura *et al.*, 2013; Hazama *et al.*, 2015; Cornu *et al.*, 2016).

Measuring Cd concentration in 69 rice accessions, Uruguchi *et al.* (2009b) found strong correlations between Cd levels in the xylem sap and in shoots and grains. Zhang *et al.* (2018) obtained similar results in the case of rape (*B. napus*). Harris and Taylor (2013) demonstrated the importance of root-to-shoot translocation in the accumulation of Cd in durum wheat grain. Together, these results demonstrate the importance of xylem transport for shoot Cd accumulation across species. However, the transpiration rate generally only slightly affects the root-to-shoot translocation of Cd (Van der Vliet *et al.*, 2007; Liñero *et al.*, 2016), probably because it is always sufficient to transport the Cd absorbed by the roots to the shoots.

C. Cadmium speciation in the xylem sap

Theoretically, based on the pH and the composition of the xylem sap, Cd could be present as a free ion or involved in various complexes with sulfhydryl (SH), or N- or O-based groups. Cadmium could be linked to cysteine-containing molecules, such as glutathione or phytochelatins, organic and amino acids (Welch and Norvell, 1999).

White *et al.* (1981) have shown that Cd forms negatively-charged complexes in soybean and tomato xylem sap. In soybean, Cataldo *et al.* (1988) found Cd as a single, slightly anionic species and as a cationic component, which appeared to be inorganic Cd. Citric acid promotes the translocation of Cd in tomato, which may be due to the formation of citrate Cd complexes in the xylem (Senden *et al.*, 1995). In contrast, in the xylem sap of *B. juncea*, Salt *et al.* (1995) found that Cd is coordinated either with oxygen or with nitrogen, which excludes a complexation by PCs or MTs. Based on the analysis of low molecular weight compounds in the xylem sap of *B. juncea*, Wei *et al.* (2007) calculated that the Cd speciation was 33% organic acid–Cd and 67% inorganic anion–Cd in plants exposed to a low Cd concentration (10^{-8} M). In the xylem of plants exposed to 10 μ M Cd, the calculated Cd speciation was 1% PC–Cd, 35% organic acid–Cd, and 64% inorganic anion–Cd.

According to Ueno *et al.* (2008), based on nuclear magnetic resonance measurement and modelling of chemical speciation, Cd is essentially present as a free ion in the xylem sap of the hyperaccumulator *A. halleri*. In castor bean plants (*Ricinus communis*) exposed to 1 μ M Cd for 3 weeks, 92% of the Cd in xylem was found to be present as a free cation (Hazama *et al.*, 2015). In sunflower exposed to low and realistic Cd concentrations (2.5 nM or 20 nM), Cornu *et al.* (2016) determined that the fraction of free Cd ion in the xylem sap was around 50%. These results are difficult to reconcile, as they suggest that depending on the species and on the Cd concentration in the medium, Cd may be transported within the xylem both as complexes with organic and inorganic acids, but also as a hydrated free cation.

894 **D. Xylem unloading and Cd distribution in shoots**

After their long-distance transport in the xylem sap towards the aerial parts of the plant, mineral ions are unloaded from the xylem vessels to be distributed in the other leaf tissues (Figure 5). Xylem unloading of Cd is expected to follow the same process as the nutrient ions. However, even for nutrients, this process has yet to be completely described (Shabala, 2007).

899 Because of water evaporation, element concentration in the xylem sap of the leaves can
 900 potentially increase strongly. To prevent toxic salt excess, plants have evolved mechanisms of
 901 xylem unloading in leaves. These mechanisms take place in the highly-branched network of
 902 major and minor veins crossing the leaf blade. The ions may then be transported from the
 903 xylem parenchyma towards leaf cells via symplastic pathways. There is very little information
 904 about the transport systems responsible for the unloading of Cd from the xylem. Cd^{2+}
 905 probably enters the leaf cells using transport proteins dedicated to nutrients (Fe, Mn, Zn), as
 906 occurs during root absorption. In the shoot of *N. caerulescens*, HMA4 and HMA3 expression
 907 is highest in the bundle sheath of the vein, while for *A. halleri*, these proteins are expressed in
 908 greater amounts in the mesophyll. However, their role in the distribution and storage of Cd
 909 (and Zn) in the shoot is unclear and the transport pathway to the epidermis, in the case of *N.*
 910 *caerulescens*, remains unknown (Mishra *et al.*, 2017).

911 Cosio *et al.* (2004) measured the Michaelis-Menten kinetic parameters (K_m , V_{max}) of Cd
 912 uptake by mesophyll protoplasts of *N. caerulescens* (Ganges and Prayon populations) and of
 913 *A. halleri*. They observed no significant difference between the three plants, although *N.*
 914 *caerulescens* Ganges and *A. halleri* were both Cd hyperaccumulators while *N. caerulescens*
 915 Prayon accumulates Cd at a lower level. The authors suggested the existence of regulation
 916 mechanisms upstream of the plasma membrane of leaf mesophyll cells to explain the
 917 differences in shoot accumulation between populations. Alternatively, Cd accumulation may
 918 be driven downstream by enhanced sequestration into vacuoles. Pre-exposure of the plants to
 919 Cd induced an increase in Cd accumulation in protoplasts of “Ganges”, whereas it reduced Cd
 920 accumulation in *A. halleri*. Cosio *et al.* (2004) suggested that several parallel transport
 921 systems could be responsible for Cd internalization. These could be the Zn and Ca uptake
 922 pathways for the Prayon accession, whilst other as yet to be described transporters would be
 923 used in the Ganges accession.

924 Cosio *et al.* (2005) showed that at very low Cd exposure, the metal was preferentially located
 925 in the young leaves of *N. caerulescens*, while at higher exposures, the metal accumulated
 926 more in the older leaves. In both Ganges and Prayon populations, Cd was present mainly at
 927 the edges of the leaves, where it was much more concentrated in the epidermal cells,
 928 particularly in the large ones, than in the mesophyll cells. The relationship between cell size
 929 and Cd concentration in the epidermis suggests that vacuolar sequestration may be a driving
 930 force for Cd distribution in leaves. Cadmium hotspots spread across the entire limb surface
 931 were also observed. These correspond to the necrotic spots visible on the leaves of the Prayon
 932 population. Cadmium is not only concentrated in vacuoles, but also in cell walls, which
 933 together account for up to 35% of the metal in the leaves (Cosio *et al.*, 2005).

934 Measuring Cd uptake kinetics from leaf sections on *N. caerulescens*, Ebbs *et al.* (2009)
 935 observed higher K_m and higher V_{max} for the leaf mesophyll cells in the Prayon accession than
 936 for that of the Ganges. Using influx measurements in various types of leaf protoplasts from
 937 the Ganges accession, Leitenmaier and Küpper (2011) confirmed that sequestration in the
 938 vacuole indeed drives Cd uptake into cells. These authors also showed that the influx into
 939 specific so-called epidermal storage cells was stronger than in the other protoplasts of the
 940 epidermis and mesophyll. This indicates that the storage of Cd in epidermal tissues is due to
 941 active Cd uptake rather than to transpiration, in contrast to what had been suggested for Indian
 942 mustard (*B. juncea*) (Salt *et al.*, 1995).

943 These results are in line with previous findings in *Silene vulgaris*. In this species, Cd
 944 preferentially accumulates in the lower epidermis, independently of the transpiration flow
 945 (Chardonens *et al.*, 1998). In *Noccaea praecox*, another Cd and Zn hyperaccumulator, the
 946 Cd concentration is twice as high in the upper and lower leaf epidermis than in the mesophyll
 947 (Vogel-Mikus *et al.*, 2008). However, because of the larger volume of the mesophyll
 948 compared with that of the epidermis, mesophyll should still be considered as a location where

949 significant amounts of Cd are detoxified. In the embryo, Cd is also preferentially stored in the
 950 epidermis of the cotyledons of *N. praecox* (Vogel-Mikus *et al.*, 2007). Studies on *N. praecox*
 951 and on *A. halleri* confirmed that vacuolar sequestration as a complex with organic acids, and
 952 binding to the cell walls are the main mechanisms allowing Cd accumulation in shoots. They
 953 also indicated that Cd-thiolate complexes would be the species transported through phloem
 954 (Vogel-Mikuš *et al.*, 2010; Huguet *et al.*, 2012).

955 Ueno *et al.* (2005) found that Cd formed complexes with malate, probably located in leaf
 956 vacuoles of *N. caerulescens* (Ganges accession). In *S. alfredii*, Cd also appeared to be stored
 957 as malate in vacuoles of parenchyma tissues (pith, cortex, and mesophyll) thanks to efficient
 958 Cd transport across the tonoplast and a constitutively high concentration of malate in the
 959 vacuoles (Tian *et al.*, 2011; Tian *et al.*, 2017). .

960 In the case of willow (*Salix viminalis*) exposed to various Cd concentrations, Cd is located at
 961 the tips and edges of the young leaves, as well as at the base of the older leaves. This
 962 localization coincides with necrotic spots (Cosio *et al.*, 2006). Vollenweider *et al.* (2006)
 963 noted that in willow leaves, the pectin-rich layers of the collenchyma cell walls of the veins
 964 are the main Cd sink. They also observed lesions in the phloem, thus suggesting that
 965 retranslocation of Cd occurs through this pathway. In the leaves of *A. thaliana*, Van
 966 Belleghem *et al.* (2007) detected Cd in tracheids, but not in the mesophyll tissue. Based on the
 967 high symplastic and apoplastic sequestration of Cd in the root parenchyma, combined with
 968 retranslocation from the leaves *via* the phloem, the authors suggested that *A. thaliana* has an
 969 excluding strategy. This strategy is probably similar in *Salix viminalis* and in all other
 970 excluders, while the strategy used by hyperaccumulators is to translocate most of the metal to
 971 the shoots, where it is stored in various tissues and cell compartments.

972 ***E. Allocation through phloem***

973 Phloem plays a key role in the distribution of Cd in the aerial parts of the plant, particularly in
 974 sink organs such fruits and seeds. For instance, Kato *et al.* (2010) showed that the Cd
 975 concentrations in the grains of three rice varieties correlates with the Cd concentration in the
 976 phloem sap, but not with that in the xylem sap. Tanaka *et al.* (2007) evaluated that 91% to
 977 100% of Cd accumulated in rice grains is transported via the phloem. Many studies of phloem
 978 flow have tried to determine the extent to which it contributes to Cd reallocation from shoots
 979 to fruits, when compared to a direct translocation from the roots.

980 Several works have showed that Cd is reallocated from leaves to fruits, grain or even roots
 981 through the phloem flow (Herren and Feller, 1997; Fismes *et al.*, 2005; Yoneyama *et al.*,
 982 2010; Wei *et al.*, 2014). Using Ca as a reference of phloem supply, Liñero *et al.* (2018)
 983 concluded that phloem is the main route for transporting Cd to sunflower seeds. They
 984 calculated that the contribution of the net remobilization from the shoot accounted for 14% of
 985 the total Cd amount in mature seeds. Using Cd stable isotopes to trace the metal fluxes in two
 986 durum wheat cultivars, Yan *et al.* (2018) showed that 50 to 60% of the Cd accumulated in
 987 mature grains is remobilized through phloem from pre-anthesis stores, i.e leaves and stem.
 988 However, the metal is poorly remobilized from the leaves, even during senescence. This led
 989 the authors to consider these organs as irreversible Cd sinks. In contrast, Cd from the stem is
 990 efficiently remobilized towards the seeds.

991 In the hyperaccumulator *S. alfredii*, Cd storage and distribution has been shown to depend on
 992 the Ca status. Calcium deficiency seems to trigger a phloem transport system that reallocates
 993 Cd from storage tissues (old leaves) to newly grown tissues (Tian *et al.*, 2016). In durum
 994 wheat, Cakmak *et al.* (2000) observed a decrease in Cd reallocation with increasing Zn
 995 concentration in the nutrient solution. To account for this interaction, they suggested a
 996 competitive inhibition of Cd loading into phloem sap by Zn.

997 In rice, the rise in Cd concentration in the leaves is much slower than the flow of xylem sap.
 998 Indeed under direct observation by ^{107}Cd positron emission, the metal failed to reach leaf
 999 blades after 36 h even though it had already reached the panicles after 7 hours of exposure
 1000 (Fujimaki *et al.*, 2010). This suggests that Cd transport is decoupled from the xylem sap flow.
 1001 In addition, Cd was singularly accumulated in the nodes and redistributed to the non-exposed
 1002 roots, thereby indicating a major role for phloem in the transport and distribution of Cd in the
 1003 plant (Fujimaki *et al.*, 2010). Kobayashi *et al.* (2013) provided evidence that Cd can be loaded
 1004 into the rice phloem at the level of first node immediately after the metal has been transported
 1005 from the root. Phloem is also involved in Cd translocation from root to leaves in *Solanum*
 1006 *melongena* and *Tagetes erecta* (Qin *et al.*, 2013; Qin *et al.*, 2015).
 1007 OsIRT1 is expressed in the rice phloem, in stem and leaves and has been suggested to
 1008 transport Fe into phloem cells (Ishimaru *et al.*, 2006). This transporter may therefore also
 1009 contribute to the loading of Cd into the phloem. OsLCT1, the rice homologue of the low
 1010 affinity cation transporter LCT1, identified in a wheat cDNA library (Clemens *et al.*, 1998) is
 1011 expressed in the nodes and leaf blades at rice reproductive stages (Table 1). This phloem Cd
 1012 transporter would appear to be responsible for Cd delivery to rice grains, probably by
 1013 mediating xylem to phloem Cd transfer in the nodes and/or re-translocation of Cd from leaves
 1014 to grains (Uraguchi *et al.*, 2011; Uraguchi *et al.*, 2014). In addition, Yamaji and Ma (2017)
 1015 suggested that OsHMA2, which is highly and constitutively expressed in the nodes, close to
 1016 phloem in vascular bundles, could also be involved in mediating Cd (and Zn) transfer from
 1017 the xylem sap to the phloem.

1018 ***F. Cadmium speciation in the phloem sap***

1019 Mendoza-Cozatl *et al.* (2008) found high ratios between PCs and Cd and between glutathione
 1020 (GSH) and Cd in the phloem sap of *B. napus*. They hypothesized that the metal could be
 1021 complexed with these compounds, which would then act as long-distance Cd carriers. Kato *et*

al. (2010) showed that, in rice phloem sap, Cd may bind to a 13 kDa protein distinct from MT. Cd is also associated to SH compounds, probably PCs and GSH. Hazama *et al.* (2015) studied the speciation of Cd in the phloem sap of the castor bean (*R. communis*). Based on size-exclusion HPLC, they found that about 50% of the metal is bound to Cd-containing proteins or peptides, mixtures of proteins and PCs (such as PC4 and PC3) as well as complexes with GSH and PC2.

X. Interaction of Cd with other elements

Cadmium absorption by roots can be affected by the other elements in the soil solution, in three ways. First, as mentioned above, some anions such as Cl^- , NO_3^- , or SO_4^- , can form complexes with Cd and modify the diffusion and the absorption of the metal. Moreover, major ions such as H^+ , Ca^{2+} and Mg^{2+} , present at concentrations several orders of magnitude higher than those of Cd, decrease the plasma membrane surface potential, by compensating the negatively-charged lipid heads present at the external side of the plasma membrane. In the absence of negative charges, which would attract them, the local concentrations of trace cations, such as Cd^{2+} , at the vicinity of the plasma membrane are reduced. The lower local concentration around the uptake transporters results in lower uptake (Kinraide, 1998; Wang *et al.*, 2011; Wang *et al.*, 2012). On the other hand, Cd^{2+} and other cations directly compete for transport. Using soybean in hydroponics, Cataldo *et al.* (1983) showed that root absorption of Cd^{2+} fell in the presence of Cu^{2+} , Fe^{2+} , Mn^{2+} , and Zn^{2+} , thus suggesting that these cations compete with Cd^{2+} to enter the root through the same transport systems. Hart *et al.* (2002) also observed that trace amounts of Zn in the exposure solution inhibit Cd uptake by roots of durum wheat (and vice versa), thereby indicating that the two ions compete for root uptake via the same transport systems. In contrast, Salt *et al.* (1997) found no competition of Mn^{2+} and Zn^{2+} with Cd^{2+} for root absorption by *B. juncea*. Sterckeman *et al.* (2011) exposed maize

1046 root plants to a 1 μM Cd solution with 5 μM of another cation (Ca^{2+} , Co^{2+} , Cu^{2+} , Fe^{2+} , Mg^{2+} ,
1047 Mn^{2+} , Na^+ , NH_4^+ , Ni^{2+} , Pb^{2+} or Zn^{2+}) and found a reduction in Cd uptake by Cu^{2+} only.

1048 The expression of many transporters of the ZIP family, such as ZNT1, is only detectable
1049 under Zn deficiency, except in Zn hyperaccumulating plants (Grotz *et al.*, 1998; Verbruggen
1050 *et al.*, 2009b). This up-regulation may account for the increase in Cd absorption observed
1051 under Zn deficiency and the lower Cd accumulation when Zn is applied as a soil or foliar
1052 fertilizer (Saifullah *et al.*, 2016; Qaswar *et al.*, 2017). Zinc can also affect Cd translocation, as
1053 these metals compete with each other for xylem loading by the metal pumping ATPase
1054 OsHMA2 in rice (Fontanili *et al.*, 2016). Such competition is likely to occur in many
1055 transporters, which are not Cd specific, such as HMA4 or CAX.

1056 There is a relationship between the Fe nutritional status of the plant and Cd accumulation
1057 (Chen *et al.*, 2019). As mentioned above, Fe deficiency induces the expression of IRT1,
1058 which is responsible for Cd absorption. The positive effect of Fe deficiency on Cd uptake was
1059 observed in *N. caerulea* and in *A. thaliana* (Lombi *et al.*, 2002; Vert *et al.*, 2002).

1060 Conversely, Cd stress induces symptoms similar to those of Fe deficiency, in particular the
1061 biosynthesis and secretion of phytosiderophores, which improve Fe uptake and provides an
1062 advantage under Cd stress relative to Fe acquisition (Astolfi *et al.*, 2014). He *et al.* (2017)
1063 showed that supplying *A. thaliana* with Fe^{2+} prevents Cd uptake by inhibiting IRT1
1064 expression and favouring Fe^{2+} in its competition with Cd^{2+} . The Fe transporter, NRAMP also
1065 transports Cd (Thomine *et al.*, 2000; Takahashi *et al.*, 2011), which suggest a possible
1066 competition between the two elements at this level (Chen *et al.*, 2019).

1067 Cadmium also interacts with the Fe plaque that is deposited at the root surface under certain
1068 conditions. An Fe plaque is commonly formed in various aquatic and terrestrial plant species,
1069 such as rice (*O. sativa*). Radial loss of oxygen from the root and oxidation by microorganisms

1070 cause the oxidation of ferrous to ferric iron, which precipitates at the root surface as iron
 1071 hydroxide (Liu *et al.*, 2007). The iron plaque is expected to sequester trace metals through
 1072 adsorption or co-precipitation, thereby affecting the availability and uptake of these elements
 1073 by the root. However, the effect of the iron plaque on Cd uptake is controversial. Liu *et al.*
 1074 (2007); Liu *et al.* (2008) found that the iron plaque is of little significance for Cd uptake and
 1075 accumulation by rice plants and suggested instead that root tissues control Cd uptake in rice.
 1076 In contrast, Cheng *et al.* (2014) and Cao *et al.* (2018b) showed that the iron plaque indeed
 1077 promotes Cd deposition onto root surface and limits metal accumulation in rice plants.
 1078 Sebastian and Prasad (2016) also suggested that iron plaque would act as a barrier against Cd
 1079 exposure, but also as an Fe source preventing Cd stress and Cd-induced Fe deficiency.
 1080 However, Zhou *et al.* (2018) found a positive correlation between Cd in iron plaque and Cd in
 1081 rice plant tissues, suggesting that the plaque does not prevent Cd accumulation by the plant.
 1082 However, the same group of authors (Zhou *et al.*, 2015) previously reported a negative
 1083 correlation between Cd in the iron plaque and that in the roots of 32 rice cultivars, suggesting
 1084 a protective role of the plaque.

1085 Nitrogen speciation also influences Cd root uptake independently of its effect on rhizosphere
 1086 or root apoplast pH (see above). Indeed, in hydroponics, supplying *N. caerulescens* with NO_3^-
 1087 resulted in a doubling of the Cd concentration in the shoots compared with NH_4^+ supply.
 1088 Moreover, when cultivating the same species in soil, Cd uptake was highest in the NO_3^-
 1089 treatment, despite the fact that a higher pH decreases Cd and Zn bioavailability in the
 1090 rhizosphere under these conditions (Xie *et al.*, 2009). Nitrate also enhances Cd uptake in
 1091 tomato (*Solanum lycopersicum*) (Luo *et al.*, 2012), potato (*S. tuberosum*) (Larsson Jönsson
 1092 and Asp, 2013) and *S. alfredii* (Hu *et al.*, 2013) grown in hydroponics. However, the
 1093 mechanisms accounting for this positive effect of NO_3^- on Cd absorption are still unclear. It
 1094 has been proposed that the uptake of NO_3^- would favour Cd uptake by increasing membrane

1095 potential, while that of NH_4^+ would depolarize the membrane (Monsant *et al.*, 2010). More
 1096 recently, based on results obtained with rice, Yang *et al.* (2016) proposed that NO_3^- enhances
 1097 Cd uptake by up-regulating the expression of IRT1. However, in the Cd accumulators *S.*
 1098 *nigrum* and *Carpobrotus rossii*, shoot Cd accumulation was 30% higher with NH_4^+ than with
 1099 NO_3^- in the nutrient solution (Cheng *et al.*, 2016).

1100 Some of the works cited above suggest that N forms can affect Cd absorption not only by the
 1101 root, but also its translocation (Hu *et al.*, 2013; Larsson Jönsson and Asp, 2013; Cheng *et al.*,
 1102 2016), through as yet unknown mechanisms. Li *et al.* (2010) reported that Cd regulates the
 1103 low affinity nitrate transporter AtNRT1.8, which controls nitrate concentration in the xylem
 1104 sap. Moreover, a loss of the AtNRT1.8 function increased Cd sensitivity, thus suggesting
 1105 cross-talk between Cd and nitrate translocation.

1106 Nocito *et al.* (2002) showed that the formation of PC-Cd complexes (see above) induces an
 1107 increase in sulphate uptake by maize, in order to sustain the higher sulphur demand during PC
 1108 synthesis. Moreover, Cd stimulates the expression of genes involved in sulphur assimilation
 1109 pathways (Harada *et al.*, 2002; Herbette *et al.*, 2006; Ernst *et al.*, 2008; Liang *et al.*, 2014;
 1110 Liang *et al.*, 2016). Wu *et al.* (2018) found that an excess supply of sulphate increases Cd
 1111 accumulation in the leaves of *Vicia faba*. In rice, Yamaguchi *et al.* (2016) showed that Cd
 1112 exposure up-regulated the transcription of the sulphate transporter SULTR1/2, as well as
 1113 sulphate uptake and translocation to shoot.

1114 Silicon (Si) is known to reduce Cd toxicity to plants (Nwugo and Huerta, 2008; Vieira da
 1115 Cunha *et al.*, 2008; Vaculík *et al.*, 2009; Wang *et al.*, 2015c; Rahman *et al.*, 2017). It was
 1116 reported to decrease the availability of Cd in soil (Liang *et al.*, 2005; Vieira da Cunha *et al.*,
 1117 2008) and decrease Cd absorption and/or translocation (Liang *et al.*, 2005; Wang *et al.*,
 1118 2015c; Wu *et al.*, 2016b; Rahman *et al.*, 2017; Shao *et al.*, 2017), although not systematically
 1119 (Vaculík *et al.*, 2009). Silicon improves Cd sequestration and complexation, restores the

1120 redox balance and strengthens the antioxidant capacity of the plant (Farooq *et al.*, 2016).
 1121 Alleviation of Cd toxicity by Si has also been attributed to enhanced binding of Cd to the root
 1122 apoplast (Vaculík *et al.*, 2012; Wu *et al.*, 2016b). More recently, the work of Greger *et al.*
 1123 (2016) on wheat (*T. aestivum*) showed that SiO₃ down-regulated LCT1 and HMA2 and up-
 1124 regulated PCS1. IRT1 was down-regulated by Cd and up-regulated by Si in roots and shoot.
 1125 Silicon had no effect on the expression of NRAMP1. Shao *et al.* (2017) showed that
 1126 OsNRAMP5 and OsHMA2 were down-regulated by Si, which could explain the reduction in
 1127 Cd uptake and translocation in rice exposed to silicic acid.

1128 **XI. Conclusions**

1129 Knowledge of Cd accumulation mechanisms in plants has considerably progressed over
 1130 recent decades. However, many uncertainties remain and this knowledge is still incomplete at
 1131 various levels. Cadmium speciation in the rhizosphere solution, which controls the root
 1132 uptake, is poorly understood, because of the difficulties in identifying and quantifying the
 1133 species, particularly the organometallic complexes. There is also a need to better characterize
 1134 the reactive sites in the soil solid phase in order to accurately predict Cd solubility.

1135 Concerning the processes responsible for the distribution of the metal in the plant tissues and
 1136 organs, much is known of the mechanisms and the factors (such as the transport systems)
 1137 controlling root absorption and storage, and xylem loading. In contrast, xylem unloading,
 1138 transport and storage in the stem and leaf tissues, as well as reallocation through phloem
 1139 loading and transport towards the reproductive organs are still poorly understood. It is likely
 1140 that future work will shed new light on the mechanisms of Cd distribution in aerial parts and
 1141 especially to fruits and seeds.

1142 The diversity of the transport systems involved in Cd uptake and translocation is consistent
 1143 with the idea that Cd, as a non-essential element, hijacks the pathways of certain nutrients,

such as calcium, zinc, manganese or iron to accumulate in plants. Moreover, the pathways used by Cd differ according to plant species. Investigations in the model species *A. thaliana* and in rice have revealed striking differences in the uptake mechanisms, as well as commonalities in the mechanisms that allow Cd sequestration in roots. There will be a need to extend this knowledge to other species, including species with contrasting accumulations and crops such as lettuce, cocoa and tobacco, for which Cd accumulation is an important public health issue. Moreover, the precise location of root Cd carriers at the tissue scale, will need to be determined in the future in order to better describe Cd uptake paths into plants.

Among crop species, rice has been the subject of recent major advances. Whilst rice produces the staple diet of hundreds of millions of people in the world, it is the cereal that accumulates Cd at the highest level in its grains. These scientific advances are about to lead to the release of rice cultivars that accumulate much less Cd. In contrast, few results have been obtained on bread wheat (*T. aestivum*) and durum wheat, whereas these cereals are responsible for the chronic exposure to Cd of a very large portion of the world population (EFSA, 2012). In the future, research should focus on the biomolecular mechanisms of Cd accumulation in wheat.

Recent works have suggested the involvement of new actors in Cd transport in plants, such as CAL1, a defensin-like protein, which would appear to chelate the metal and favour its xylem loading, or again the homeodomain-containing protein gene, OsHB4a, which regulates several genes involved in Cd uptake and translocation. The regulation of OsHB4a by the miR166 also indicates that other mechanisms involving micro-RNA may be important for the movement of Cd in a plant. The effects of Si or of N forms on Cd accumulation are relatively new discoveries. These correspond to complex processes, which are as yet only partly explained, but may help to improve the management of Cd-contaminated land. All these topics represent avenues of research leading to the possibility of reducing Cd accumulation in crops.

1173 **References**

- 1174 Alloway, B. J. 1995. Cadmium. In: Alloway, B. J. (ed) Heavy metals in soils. Second edn.
 1175 Blackie Academic & Professional, Glasgow.
- 1176 Arnold, T., Kirk, G. J. D., Wissuwa, M., Frei, M., Zhao, F.-J., Mason, T. F. D. and Weiss, D.
 1177 J. 2010. Evidence for the mechanisms of zinc uptake by rice using isotope
 1178 fractionation. *Plant Cell Environ.* **33**: 370-381.
- 1179 Arrivault, S., Senger, T. and Krämer, U. 2006. The Arabidopsis metal tolerance protein
 1180 AtMTP3 maintains metal homeostasis by mediating Zn exclusion from the shoot
 1181 under Fe deficiency and Zn oversupply. *Plant J.* **46**: 861-879.
- 1182 Astolfi, S., Ortolani, M. R., Catarcione, G., Paolacci, A. R., Cesco, S., Pinton, R. and Ciaffi,
 1183 M. 2014. Cadmium exposure affects iron acquisition in barley (*Hordeum vulgare*)
 1184 seedlings. *Physiol. Plant.* **152**: 646-659.
- 1185 ATSDR 2012. Toxicological profile for cadmium. U.S. Department of Health and Human
 1186 Services, Public Health Service, Agency for Toxic Substances and Disease Registry,
 1187 Atlanta.
- 1188 Baker, A. J. M. 1981. Accumulators and excluders - strategies in the response of plants to
 1189 heavy metals. *J. Plant Nutr.* **3**: 643-654.
- 1190 Baliardini, C., Meyer, C.-L., Salis, P., Saumitou-Laprade, P. and Verbruggen, N. 2015.
 1191 *CATION EXCHANGER1* cosegregates with cadmium tolerance in the metal
 1192 hyperaccumulator *Arabidopsis halleri* and plays a role in limiting oxidative stress in
 1193 *Arabidopsis* Spp. *Plant Physiol.* **169**: 549-559.
- 1194 Barberon, M., Dubeaux, G., Kolb, C., Isono, E., Zelazny, E. and Vert, G. 2014. Polarization
 1195 of IRON-REGULATED TRANSPORTER 1 (IRT1) to the plant-soil interface plays
 1196 crucial role in metal homeostasis. *Proc. Natl. Acad. Sci.* **111**: 8293-8298.

- 1197 Benatti, M. R., Yookongkaew, N., Meetam, M., Guo, W.-J., Punyasuk, N., AbuQamar, S. and
 1198 Goldsbrough, P. 2014. Metallothionein deficiency impacts copper accumulation and
 1199 redistribution in leaves and seeds of *Arabidopsis*. *New Phytol.* **202**: 940-951.
- 1200 Berkelaar, E. J. and Hale, B. A. 2003a. Accumulation of cadmium by durum wheat roots:
 1201 Bases for citrate-mediated exceptions to the free ion model. *Environ. Toxicol. Chem.*
 1202 **22**: 1155-1161.
- 1203 Berkelaar, E. J. and Hale, B. A. 2003b. Cadmium accumulation by durum wheat roots in
 1204 ligand-buffered hydroponic culture: uptake of Cd–ligand complexes or enhanced
 1205 diffusion? *Can. J. Bot.* **81**: 755-763.
- 1206 Blaylock, M. J., Salt, D. E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y.,
 1207 Ensley, B. D. and Raskin, I. 1997. Enhanced accumulation of Pb in Indian mustard by
 1208 soil-applied chelating agents. *Environ. Sci. Technol.* **31**: 860-865.
- 1209 Brunetti, P., Zanella, L., De Paolis, A., Di Litta, D., Cecchetti, V., Falasca, G., Barbieri, M.,
 1210 Altamura, M. M., Costantino, P. and Cardarelli, M. 2015. Cadmium-inducible
 1211 expression of the ABC-type transporter AtABCC3 increases phytochelatin-mediated
 1212 cadmium tolerance in *Arabidopsis*. *J. Exp. Bot.* **66**: 3815-3829.
- 1213 Cailliatte, R., Schikora, A., Briat, J.-F., Mari, S. and Curie, C. 2010. High-affinity manganese
 1214 uptake by the metal transporter Nramp1 is essential for *Arabidopsis* growth in low
 1215 manganese conditions. *Plant Cell* **22**: 904-917.
- 1216 Cakmak, I., Welch, R. M., Erenoglu, B., Römheld, V., Norvell, W. A. and Kochian, L. V.
 1217 2000. Influence of varied zinc supply on re-translocation of cadmium (^{109}Cd) and
 1218 rubidium (^{86}Rb) applied on mature leaf of durum wheat seedlings. *Plant Soil* **219**:
 1219 279-284.

- 1220 Cao, D.-J., Yang, X., Geng, G., Wan, X.-C., Ma, R.-X., Zhang, Q. and Liang, Y.-G. 2018a.
 1221 Absorption and subcellular distribution of cadmium in tea plant (*Camellia sinensis* cv.
 1222 “Shuchazao”). *Environ. Sci. Pollut. Res.* **25**: 15357-15367.
- 1223 Cao, Z.-Z., Qin, M.-L., Lin, X.-Y., Zhu, Z.-W. and Chen, M.-X. 2018b. Sulfur supply reduces
 1224 cadmium uptake and translocation in rice grains (*Oryza sativa* L.) by enhancing iron
 1225 plaque formation, cadmium chelation and vacuolar sequestration. *Environ. Pollut.*
 1226 **238**: 76-84.
- 1227 Carrier, P., Baryla, A. and Havaux, M. 2003. Cadmium distribution and microlocalization in
 1228 oilseed rape (*Brassica napus*) after long-term growth on cadmium-contaminated soil.
 1229 *Planta* **216**: 939-950.
- 1230 Cataldo, D. A., Garland, T. R. and Wildung, R. E. 1983. Cadmium uptake kinetics in intact
 1231 soybean plants. *Plant Physiol.* **73**: 844-848.
- 1232 Cataldo, D. A., McFadden, K. M., Garland, T. R. and Wildung, R. E. 1988. Organic
 1233 constituents and complexation of nickel(II), iron(III), cadmium(II), and plutonium(IV)
 1234 in soybean xylem exudates. *Plant Physiol.* **86**: 734-739.
- 1235 Chaney, R. L., Reeves, H. W., Baklanov, I. A., Centofanti, T., Broadhurst, C. L., Baker, A. J.
 1236 M., Van der Ent, A. and Rosenberg, R. 2014. Phytoremediation and Phytomining:
 1237 Using plants to remediate contaminated or mineralized environments. In: Rajakaruna,
 1238 R., Boyd, R. S. and Harris, T. (eds) Plant Ecology and Evolution in Harsh
 1239 Environments. Nova Science Publishers, New York.
- 1240 Chao, D.-Y., Silva, A., Baxter, I., Huang, Y. S., Nordborg, M., Danku, J., Lahner, B.,
 1241 Yakubova, E. and Salt, D. E. 2012. Genome-wide association studies identify heavy
 1242 metal ATPase3 as the primary determinant of natural variation in leaf cadmium in
 1243 *Arabidopsis thaliana*. *PLOS Genetics* **8**: e1002923.

- 1244 Chardonens, A. N., Bookum, W. M. T., Kuijper, L. D. J., Verkleij, J. A. C. and Ernst, W. H.
 1245 O. 1998. Distribution of cadmium in leaves of cadmium tolerant and sensitive
 1246 ecotypes of *Silene vulgaris*. *Physiol. Plant.* **104**: 75-80.
- 1247 Chekmeneva, E., Díaz-Cruz, J. M., Ariño, C. and Esteban, M. 2007. Binding of Cd²⁺ and Zn²⁺
 1248 with the phytochelatin (γ-Glu-Cys)4-Gly: A voltammetric study assisted by
 1249 multivariate curve resolution and electrospray ionization mass spectrometry.
 1250 *Electroanal.* **19**: 310-317.
- 1251 Chekmeneva, E., Prohens, R., Díaz-Cruz, J. M., Ariño, C. and Esteban, M. 2008. Competitive
 1252 binding of Cd and Zn with the phytochelatin (γ-Glu-Cys)4-Gly: Comparative study
 1253 by mass spectrometry, voltammetry-multivariate curve resolution, and isothermal
 1254 titration calorimetry. *Environ. Sci. Technol.* **42**: 2860-2866.
- 1255 Chen, A., Komives, E. A. and Schroeder, J. I. 2006. An improved grafting technique for
 1256 mature arabidopsis plants demonstrates long-distance shoot-to-root transport of
 1257 phytochelatins in Arabidopsis. *Plant Physiol.* **141**: 108-120.
- 1258 Chen, C., Cao, Q., Jiang, Q., Li, J., Yu, R. and Shi, G. 2019. Comparative transcriptome
 1259 analysis reveals gene network regulating cadmium uptake and translocation in peanut
 1260 roots under iron deficiency. *BMC Plant Biol.* **19**: 35.
- 1261 Chen, H., Zhang, W., Yang, X., Wang, P., McGrath, S. P. and Zhao, F.-J. 2018a. Effective
 1262 methods to reduce cadmium accumulation in rice grain. *Chemosphere* **207**: 699-707.
- 1263 Chen, X., Ouyang, Y., Fan, Y., Qiu, B., Zhang, G. and Zeng, F. 2018b. The pathway of
 1264 transmembrane cadmium influx via calcium-permeable channels and its spatial
 1265 characteristics along rice root. *J. Exp. Bot.* **69**: 5279-5291.
- 1266 Cheng, H., Wang, M., Wong, M. and Ye, Z. 2014. Does radial oxygen loss and iron plaque
 1267 formation on roots alter Cd and Pb uptake and distribution in rice plant tissues? *Plant*
 1268 *Soil* **375**: 137-148.

- 1269 Cheng, M., Wang, P., Kopittke, P. M., Wang, A., Sale, P. W. G. and Tang, C. 2016.
 1270 Cadmium accumulation is enhanced by ammonium compared to nitrate in two
 1271 hyperaccumulators, without affecting speciation. *J. Exp. Bot.* **67**: 5041-5050.
- 1272 Chiang, H. C., Lo, J. C. and Yeh, K. C. 2006. Genes associated with heavy metal tolerance
 1273 and accumulation in Zn/Cd hyperaccumulator *Arabidopsis halleri*: a genomic survey
 1274 with cDNA microarray. *Environ. Sci. Technol.* **40**: 6792-6798.
- 1275 Choppala, G., Saifullah, Bolan, N., Bibi, S., Iqbal, M., Rengel, Z., Kunhikrishnan, A.,
 1276 Ashwath, N. and Ok, Y. S. 2014. Cellular mechanisms in higher plants governing
 1277 tolerance to cadmium toxicity. *Crit. Rev. Plant Sci.* **33**: 374-391.
- 1278 Christensen, T. H. 1984. Cadmium soil sorption at low concentrations: I. Effect of time,
 1279 cadmium load, pH, and calcium. *Water Air Soil Pollut.* **21**: 105-114.
- 1280 Cieslinski, G., Van Rees, K. C. J., Szmigielska, A. M., Krishnamurti, G. S. R. and Huang, P.
 1281 M. 1998. Low-molecular-weight organic acids in rhizosphere soils of durum wheat
 1282 and their effect on cadmium bioaccumulation. *Plant Soil* **203**: 109-117.
- 1283 Clemens, S. 2001. Molecular mechanisms of plant metal tolerance and homeostasis. *Planta*
 1284 **212**: 475-486.
- 1285 Clemens, S. 2006. Toxic metal accumulation, responses to exposure and mechanisms
 1286 of tolerance in plants. *Biochim.* **88**: 1707-1719.
- 1287 Clemens, S., Aarts, M. G. M., Thomine, S. and Verbruggen, N. 2013. Plant science: the key to
 1288 preventing slow cadmium poisoning. *Trends Plant Sci.* **18**: 92-99.
- 1289 Clemens, S., Antosiewicz, D. M., Ward, J. M., Schachtman, D. P. and Schroeder, J. I. 1998.
 1290 The plant cDNA LCT1 mediates the uptake of calcium and cadmium in yeast. *Proc.*
 1291 *Natl. Acad. Sci. U.S.A.* **95**: 12043-12048.
- 1292 Clemens, S., Kim, E. J., Neumann, D. and Schroeder, J. I. 1999. Tolerance to toxic metals by
 1293 a gene of phytochelatin synthase from plants and yeast. *EMBO J.* **18**: 3325-3333.

- 1294 Cobbett, C. and Goldsbrough, P. 2002. Phytochelatins and metallothioneins: Roles in heavy
1295 metal detoxification and homeostasis. *Annu. Rev. Plant Biol.* **53**: 159-182.
- 1296 Cobbett, C. S. 2003. Heavy metals and plants - model systems and hyperaccumulators. *New*
1297 *Phytol.* **159**: 289-293.
- 1298 Cohen, C. K., Fox, T. C., Garvin, D. F. and Kochian, L. V. 1998. The role of iron-deficiency
1299 stress responses in stimulating heavy-metal transport in plants. *Plant Physiol.* **116**:
1300 1063-1072.
- 1301 Collins, R. N., Merrington, G., McLaughlin, M. J. and Knudsen, C. 2002. Uptake of intact
1302 zinc-ethylenediaminetetraacetic acid from soil is dependent on plant species and
1303 complex concentration. *Environ. Toxicol. Chem.* **21**: 1940-1945.
- 1304 Cornu, J. Y., Bakoto, R., Bonnard, O., Bussière, S., Coriou, C., Sirguy, C., Sterckeman, T.,
1305 Thunot, S., Visse, M. I. and Nguyen, C. 2016. Cadmium uptake and partitioning
1306 during the vegetative growth of sunflower exposed to low Cd²⁺ concentrations in
1307 hydroponics. *Plant Soil* **404**: 263-275.
- 1308 Cosio, C., DeSantis, L., Frey, B., Diallo, S. and Keller, C. 2005. Distribution of cadmium in
1309 leaves of *Thlaspi caerulescens*. *J. Exp. Bot.* **56**: 765-775.
- 1310 Cosio, C., Martinoia, E. and Keller, C. 2004. Hyperaccumulation of cadmium and zinc in
1311 *Thlaspi caerulescens* and *Arabidopsis halleri* at the leaf cellular level. *Plant Physiol.*
1312 **134**: 716-725.
- 1313 Cosio, C., Vollenweider, P. and Keller, C. 2006. Localization and effects of cadmium in
1314 leaves of a cadmium-tolerant willow (*Salix viminalis* L.): I. Macrolocalization and
1315 phytotoxic effects of cadmium. *Environ. Exp. Bot.* **58**: 64-74.
- 1316 Costa, G. and Morel, J. L. 1993. Cadmium uptake by *Lupinus albus* (L.): Cadmium excretion,
1317 a possible mechanism of cadmium tolerance. *J. Plant Nutr.* **16**: 1921-1929.

- 1318 Costa, G. and Morel, J. L. 1994. Efficiency of H⁺-ATPase activity on cadmium uptake by four
1319 cultivars of lettuce. *J. Plant Nutr.* **17**: 627-637.
- 1320 Courbot, M., Willems, G., Motte, P., Arvidsson, S., Roosens, N., Saumitou-Laprade, P. and
1321 Verbruggen, N. 2007. A major quantitative trait locus for cadmium tolerance in
1322 *Arabidopsis halleri* colocalizes with HMA4, a gene encoding a heavy metal ATPase.
1323 *Plant Physiol.* **144**: 1052-1065.
- 1324 Craciun, A. R., Meyer, C.-L., Chen, J., Roosens, N., De Groodt, R., Hilson, P. and
1325 Verbruggen, N. 2012. Variation in HMA4 gene copy number and expression among
1326 *Noccaea caerulescens* populations presenting different levels of Cd tolerance and
1327 accumulation. *J. Exp. Bot.* **63**: 4179-4189.
- 1328 Cruz, B. H., Diaz-Cruz, J. M., Arino, C. and Esteban, M. 2005. Complexation of heavy metals
1329 by phytochelatin: Voltametric study of the binding of Cd²⁺ and Zn²⁺ ions by the
1330 phytochelatin (γ-Glu-Cys)₃Gly assisted by multivariate curve resolution. *Environ. Sci.*
1331 *Technol.* **39**: 778-786.
- 1332 Custos, J.-M., Moyne, C., Treillon, T. and Sterckeman, T. 2014. Contribution of Cd-EDTA
1333 complexes to cadmium uptake by maize: a modelling approach. *Plant Soil* **374**: 497-
1334 512.
- 1335 de Meeûs, C., Eduljee, G. H. and Hutton, M. 2002. Assessment and management of risks
1336 arising from exposure to cadmium in fertilisers. I. *Sci. Total Environ.* **291**: 167-187.
- 1337 Degryse, F., Buekers, J. and Smolders, E. 2004. Radio-labile cadmium and zinc in soils as
1338 affected by pH and source of contamination. *Eur. J. Soil Sci.* **55**: 113-122.
- 1339 Degryse, F., Smolders, E. and Merckx, R. 2006. Labile Cd complexes increase Cd availability
1340 to plants. *Environ. Sci. Technol.* **40**: 830-836.

- 1341 Degryse, F., Smolders, E. and Parker, D. R. 2009. Partitioning of metals (Cd, Co, Cu, Ni, Pb,
1342 Zn) in soils: concepts, methodologies, prediction and applications: a review. *Eur. J.*
1343 *Soil Sci.* **60**: 590-612.
- 1344 Desbrosses-Fonrouge, A.-G., Voigt, K., Schröder, A., Arrivault, S., Thomine, S. and Krämer,
1345 U. 2005. *Arabidopsis thaliana* MTP1 is a Zn transporter in the vacuolar membrane
1346 which mediates Zn detoxification and drives leaf Zn accumulation. *FEBS Lett.* **579**:
1347 4165-4174.
- 1348 Ding, Y., Gong, S., Wang, Y., Wang, F., Bao, H., Sun, J., Cai, C., Yi, K., Chen, Z. and Zhu,
1349 C. 2018. MicroRNA166 modulates cadmium tolerance and accumulation in rice. *Plant*
1350 *Physiol.* **177**: 1691-1703.
- 1351 Dräger, D. B., Desbrosses-Fonrouge, A.-G., Krach, C., Chardonens, A. N., Meyer, R. C.,
1352 Saumitou-Laprade, P. and Krämer, U. 2004. Two genes encoding *Arabidopsis halleri*
1353 MTP1 metal transport proteins co-segregate with zinc tolerance and account for high
1354 MTP1 transcript levels. *Plant J.* **39**: 425-439.
- 1355 Ebbs, S. D., Zambrano, M. C., Spiller, S. M. and Newville, M. 2009. Cadmium sorption,
1356 influx, and efflux at the mesophyll layer of leaves from ecotypes of the Zn/Cd
1357 hyperaccumulator *Thlaspi caerulescens*. *New Phytol.* **181**: 626-636.
- 1358 EFSA 2012. Cadmium dietary exposure in the European population. *EFSA J.* **10**: 2551.
- 1359 Eide, D., Broderius, M., Fett, J. and Guerinot, M. L. 1996. A novel iron-regulated metal
1360 transporter from plants identified by functional expression in yeast. *PNAS* **93**: 5624-
1361 5628.
- 1362 Ernst, W. H. O., Krauss, G.-J., Verkleij, J. A. C. and Wesenberg, D. 2008. Interaction of
1363 heavy metals with the sulphur metabolism in angiosperms from an ecological point of
1364 view. *Plant Cell Environ.* **31**: 123-143.

- 1365 Farooq, M. A., Detterbeck, A., Clemens, S. and Dietz, K.-J. 2016. Silicon-induced
1366 reversibility of cadmium toxicity in rice. *J. Exp. Bot.* **67**: 3573-3585.
- 1367 Farrell, R. E., McArthur, D. F. E. and Van Rees, K. C. J. 2005. Net Cd²⁺ flux at the root
1368 surface of durum wheat (*Triticum turgidum* L. var. durum) cultivars in relation to
1369 cultivar differences in Cd accumulation. *Can. J. Plant Sci.* **85**: 103-107.
- 1370 Feng, J., Jia, W., Lv, S., Bao, H., Miao, F., Zhang, X., Wang, J., Li, J., Li, D., Zhu, C., Li, S.
1371 and Li, Y. 2018. Comparative transcriptome combined with morpho-physiological
1372 analyses revealed key factors for differential cadmium accumulation in two
1373 contrasting sweet sorghum genotypes. *Plant Biotechnol. J.* **16**: 558-571.
- 1374 Fismes, J., Echevarria, G., Leclerc-Cessac, E. and Morel, J. L. 2005. Uptake and transport of
1375 radioactive nickel and cadmium into three vegetables after wet aerial contamination. *J.*
1376 *Environ. Quality* **34**: 1497-1507.
- 1377 Florijn, P. J. and Van Beusichem, M. L. 1993a. Cadmium distribution in maize inbred lines:
1378 Effects of pH and level of Cd supply. *Plant Soil* **153**: 79-84.
- 1379 Florijn, P. J. and Van Beusichem, M. L. 1993b. Uptake and distribution of cadmium in maize
1380 inbred lines. *Plant Soil* **150**: 25-32.
- 1381 Fontanili, L., Lancilli, C., Suzui, N., Dendena, B., Yin, Y.-G., Ferri, A., Ishii, S., Kawachi, N.,
1382 Lucchini, G., Fujimaki, S., Sacchi, G. A. and Nocito, F. F. 2016. Kinetic analysis of
1383 zinc/cadmium reciprocal competitions suggests a possible Zn-insensitive pathway for
1384 root-to-shoot cadmium translocation in rice. *Rice* **9**: 16.
- 1385 Fu, X., Dou, C., Chen, Y., Chen, X., Shi, J., Yu, M. and Xu, J. 2011. Subcellular distribution
1386 and chemical forms of cadmium in *Phytolacca americana* L. *J. Hazardous Mater.*
1387 **186**: 103-107.
- 1388 Fujimaki, S., Suzui, N., Ishioka, N. S., Kawachi, N., Ito, S., Chino, M. and Nakamura, S.-I.
1389 2010. Tracing cadmium from culture to spikelet: noninvasive imaging and quantitative

- 1390 characterization of absorption, transport, and accumulation of cadmium in an intact
1391 rice plant. *Plant Physiol.* **152**: 1796-1806.
- 1392 Gasic, K. and Korban, S. S. 2007. Expression of Arabidopsis phytochelatase in Indian
1393 mustard (*Brassica juncea*) plants enhances tolerance for Cd and Zn. *Planta* **225**: 1277-
1394 1285.
- 1395 Gong, J.-M., Lee, D. A. and Schroeder, J. I. 2003. Long-distance root-to-shoot transport of
1396 phytochelatin and cadmium in *Arabidopsis*. *Proc. Natl. Acad. Sci.* **100**: 10118-10123.
- 1397 Gonneau, C., Genevois, N., Frérot, H., Sirguey, C. and Sterckeman, T. 2014. Variation of
1398 trace metal accumulation, major nutrient uptake and growth parameters and their
1399 correlations in 22 populations of *Noccaea caerulescens*. *Plant Soil* **384**: 271-287.
- 1400 Greger, M., Kabir, A. H., Landberg, T., Maity, P. J. and Lindberg, S. 2016. Silicate reduces
1401 cadmium uptake into cells of wheat. *Environ. Pollut.* **211**: 90-97.
- 1402 Greger, M. and Lofstedt, M. 2004. Comparison of uptake and distribution of cadmium in
1403 different cultivars of bread and durum wheat. *Crop Sci.* **44**: 501-507.
- 1404 Grignon, C. and Sentenac, H. 1991. pH and ionic conditions in the apoplast. *Annu. Rev. Plant*
1405 *Physiol. Plant Mol. Biol.* **42**: 103-128.
- 1406 Grill, E., Löffler, S., Winnacker, E.-L. and Zenk, M. H. 1989. Phytochelatin, the heavy-
1407 metal-binding peptides of plants, are synthesized from glutathione by a specific γ -
1408 glutamylcysteine dipeptidyl transpeptidase (phytochelatase). *Proc. Natl. Acad.*
1409 *Sci.* **86**: 6838-6842.
- 1410 Grill, E., Winnacker, E.-L. and Zenk, M. H. 1985. Phytochelatin: The principal heavy-metal
1411 complexing peptides of higher plants. *Sci.* **230**: 674-676.
- 1412 Grotz, N., Fox, T., Connolly, E., Park, W., Guerinot, M. L. and Eide, D. 1998. Identification
1413 of a family of zinc transporter genes from *Arabidopsis* that respond to zinc deficiency.
1414 *Proc. Natl. Acad. Sci.* **95**: 7220-7224.

- 1415 Gu, X. and Evans, L. J. 2008. Surface complexation modelling of Cd(II), Cu(II), Ni(II), Pb(II)
1416 and Zn(II) adsorption onto kaolinite. *Geochim. Cosmochim. Acta* **72**: 267-276.
- 1417 Gu, X., Evans, L. J. and Barabash, S. J. 2010. Modeling the adsorption of Cd (II), Cu (II), Ni
1418 (II), Pb (II) and Zn (II) onto montmorillonite. *Geochim. Cosmochim. Acta* **74**: 5718-
1419 5728.
- 1420 Guo, W.-J., Meetam, M. and Goldsbrough, P. B. 2008. Examining the specific contributions
1421 of individual *Arabidopsis* metallothioneins to copper distribution and metal tolerance.
1422 *Plant Physiol.* **146**: 1697-1706.
- 1423 Ha, S.-B., Smith, A. P., Howden, R., Dietrich, W. M., Bugg, S., O'Connell, M. J.,
1424 Goldsbrough, P. B. and Cobbett, C. S. 1999. Phytochelatin synthase genes from
1425 *Arabidopsis* and the yeast *Schizosaccharomyces pombe*. *Plant Cell* **11**: 1153-1163.
- 1426 Haghiri, F. 1973. Cadmium uptake by plants. *J. Environ. Quality* **2**: 93-95.
- 1427 Hanikenne, M., Talke, I. N., Haydon, M. J., Lanz, C., Nolte, A., Motten, P., Kroymann, J.,
1428 Weigel, D. and Krämer, U. 2008. Evolution of metal hyperaccumulation required cis-
1429 regulatory changes and triplication of HMA4. *Nature* **453**: 391-396.
- 1430 Hao, X., Zeng, M., Wang, J., Zeng, Z., Dai, J., Xie, Z., Yang, Y., Tian, L., Chen, L. and Li, D.
1431 2018. A node-expressed transporter OsCCX2 Is involved in grain cadmium
1432 accumulation of rice. *Frontiers Plant Sci.* **9**.
- 1433 Harada, E., Yamaguchi, Y., Koizumi, N. and Hiroshi, S. 2002. Cadmium stress induces
1434 production of thiol compounds and transcripts for enzymes involved in sulfur
1435 assimilation pathways in *Arabidopsis*. *J. Plant Physiol.* **159**: 445-448.
- 1436 Harris, N. and Taylor, G. 2013. Cadmium uptake and partitioning in durum wheat during
1437 grain filling. *BMC Plant Biol.* **13**: 103.

- 1438 Hart, J. J., Welch, R. M., Norvell, W. A. and Kochian, L. V. 2002. Transport interactions
1439 between cadmium and zinc in roots of bread and durum wheat seedlings. *Physiol.*
1440 *Plant.* **116**: 73-78.
- 1441 Hart, J. J., Welch, R. M., Norvell, W. A., Sullivan, L. A. and Kochian, L. V. 1998.
1442 Characterization of cadmium binding, uptake, and translocation in intact seedlings of
1443 bread and durum wheat cultivars. *Plant Physiol.* **116**: 1413-1420.
- 1444 Hassinen, V. H., Tervahauta, A. I., Halimaa, P., Plessl, M., Peräniemi, S., Schat, H., Aarts, M.
1445 G. M., Servomaa, K. and Kärenlampi, S. O. 2007. Isolation of Zn-responsive genes
1446 from two accessions of the hyperaccumulator plant *Thlaspi caerulescens*. *Planta*
1447 **V225**: 977-989.
- 1448 Haynes, R. J. 1980. Ion exchange properties of roots and ionic interactions within the root
1449 apoplasm: Their role in ion accumulation by plants. *Bot. Rev.* **46**: 75-99.
- 1450 Hazama, K., Nagata, S., Fujimori, T., Yanagisawa, S. and Yoneyama, T. 2015.
1451 Concentrations of metals and potential metal-binding compounds and speciation of
1452 Cd, Zn and Cu in phloem and xylem saps from castor bean plants (*Ricinus communis*)
1453 treated with four levels of cadmium. *Physiol. Plant.* **154**: 243-255.
- 1454 He, J., Qin, J., Long, L., Ma, Y., Li, H., Li, K., Jiang, X., Liu, T., Polle, A., Liang, Z. and
1455 Luo, Z.-B. 2011. Net cadmium flux and accumulation reveal tissue-specific oxidative
1456 stress and detoxification in *Populus × canescens*. *Physiol. Plant.* **143**: 50-63.
- 1457 He, X. L., Fan, S. K., Zhu, J., Guan, M. Y., Liu, X. X., Zhang, Y. S. and Jin, C. W. 2017. Iron
1458 supply prevents Cd uptake in Arabidopsis by inhibiting IRT1 expression and favoring
1459 competition between Fe and Cd uptake. *Plant Soil* **416**: 453-462.
- 1460 Helmke, P. A. 1999. Chemistry of cadmium in soil solution. In: McLaughlin, M. J. and Singh,
1461 B. R. (eds) Cadmium in soils and plants. Kluwer Academic Publishers, Dordrecht.

- Herbette, S., Taconnat, L., Hugouvieux, V., Piette, L., Magniette, M. L. M., Cuine, S., Auroy, P., Richaud, P., Forestier, C., Bourguignon, J., Renou, J. P., Vavasseur, A. and Leonhardt, N. 2006. Genome-wide transcriptome profiling of the early cadmium response of *Arabidopsis* roots and shoots. *Biochim.* **88**: 1751-1765.
- Hernandez-Allica, J., Garbisu, C., Becerril, J. M., Barrutia, O., Garcia-Plazaola, J. I., Zhao, F. J. and McGrath, S. P. 2006. Synthesis of low molecular weight thiols in response to Cd exposure in *Thlaspi caerulescens*. *Plant Cell Environ.* **29**: 1422-1429.
- Herren, T. and Feller, U. 1997. Transport of cadmium via xylem and phloem in maturing wheat shoots: Comparison with the translocation of zinc, strontium and rubidium. *Ann. Bot.* **80**: 623-628.
- Hill, K. A., Lion, L. W. and Ahner, B. A. 2002. Reduced Cd accumulation in *Zea mays*: A protective role for phytosiderophores? *Environ. Sci. Technol.* **36**: 5363-5368.
- Hinesly, T. D., Alexander, D. E., Ziegler, E. L. and Barrett, G. L. 1978. Zinc and Cd accumulation by corn inbreds grown on sludge amended soil. *Agron. J.* **70**: 425-428.
- Hinsinger, P., Plassard, C., Tang, C. and Jaillard, B. 2003. Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: A review. *Plant Soil* **248**: 43-59.
- Hirschi, K. D., Korenkov, V. D., Wilganowski, N. L. and Wagner, G. J. 2000. Expression of *Arabidopsis* CAX2 in Tobacco. Altered metal accumulation and increased manganese tolerance. *Plant Physiol.* **124**: 125-134.
- Holm, P. E., Andersen, B. B. H. and Christensen, T. H. 1996. Cadmium solubility in aerobic soils. *Soil Sci. Soc. Am. J.* **60**: 775-780.
- Honjo, M. N. and Kudoh, H. 2019. *Arabidopsis halleri*: a perennial model system for studying population differentiation and local adaptation. *AoB Plants* **11**.

- 1486 Hu, P., Yin, Y.-G., Ishikawa, S., Suzui, N., Kawachi, N., Fujimaki, S., Igura, M., Yuan, C.,
 1487 Huang, J., Li, Z., Makino, T., Luo, Y., Christie, P. and Wu, L. 2013. Nitrate facilitates
 1488 cadmium uptake, transport and accumulation in the hyperaccumulator *Sedum*
 1489 *plumbizincicola*. *Environ. Sci. Pollut. Res.* **20**: 6306-6316.
- 1490 Huguet, S., Bert, V., Laboudigue, A., Barthès, V., Isaure, M.-P., Llorens, I., Schat, H. and
 1491 Sarret, G. 2012. Cd speciation and localization in the hyperaccumulator *Arabidopsis*
 1492 *halleri*. *Environ. Exp. Bot.* **82**: 54-65.
- 1493 Hutchinson, J. J., Young, S. D., McGrath, S. P., West, H. M., Black, C. R. and Baker, A. J. M.
 1494 2000. Determining uptake of 'non-labile' soil cadmium by *Thlaspi caerulescens* using
 1495 isotopic dilution techniques. *New Phytol.* **146**: 453-460.
- 1496 Ishikawa, S., Ishimaru, Y., Igura, M., Kuramata, M., Abe, T., Senoura, T., Hase, Y., Arao, T.,
 1497 Nishizawa, N. K. and Nakanishi, H. 2012. Ion-beam irradiation, gene identification,
 1498 and marker-assisted breeding in the development of low-cadmium rice. *Proc. Natl.*
 1499 *Acad. Sci.* **109**: 19166-19171.
- 1500 Ishimaru, Y., Suzuki, M., Tsukamoto, T., Suzuki, K., Nakazono, M., Kobayashi, T., Wada,
 1501 Y., Watanabe, S., Matsubashi, S., Takahashi, M., Nakanishi, H., Mori, S. and
 1502 Nishizawa, N. K. 2006. Rice plants take up iron as an Fe^{3+} phytosiderophore and as
 1503 Fe^{2+} . *Plant J.* **45**: 335-346.
- 1504 Ishimaru, Y., Takahashi, R., Bashir, K., Shimo, H., Senoura, T., Sugimoto, K., Ono, K.,
 1505 Yano, M., Ishikawa, S., Arao, T., Nakanishi, H. and Nishizawa, N. K. 2012.
 1506 Characterizing the role of rice NRAMP5 in manganese, iron and cadmium transport.
 1507 *Sci. Rep.* **2**: 286.
- 1508 Jack, E., Hakvoort, H. W. J., Reumer, A., Verkleij, J. A. C., Schat, H. and Ernst, W. H. O.
 1509 2007. Real-time PCR analysis of metallothionein-2b expression in metallicolous and

- 1510 non-metallicolous populations of *Silene vulgaris* (Moench) Garcke. *Environ. Exp. Bot.*
 1511 **59**: 84-91.
- 1512 Jacquart, A., Brayner, R., El Hage Chahine, J.-M. and Ha-Duong, N.-T. 2017. Cd²⁺ and Pb²⁺
 1513 complexation by glutathione and the phytochelatins. *Chemico-Biol. Interactions* **267**:
 1514 2-10.
- 1515 Jarvis, S. C., Jones, L. H. P. and Hopper, M. J. 1976. Cadmium uptake from solution by
 1516 plants and its transport from roots to shoots. *Plant Soil* **44**: 179-191.
- 1517 Jiang, R. F., Ma, D. Y., Zhao, F. J. and McGrath, S. P. 2005. Cadmium hyperaccumulation
 1518 protects *Thlaspi caerulescens* from leaf feeding damage by thrips (*Frankliniella*
 1519 *occidentalis*). *New Phytol.* **167**: 805-814.
- 1520 John, M. K. 1973. Cadmium uptake by eight food crops as influenced by various soil levels of
 1521 cadmium. *Environ. Pollut.* **4**: 7-15.
- 1522 John, M. K., Chuah, H. H. and VanLaerhoven, C. 1972. Cadmium contamination of soil and
 1523 its uptake by oats. *Environ. Sci. Technol.* **6**: 555-557.
- 1524 Jones, D., Nguyen, C. and Finlay, R. 2009. Carbon flow in the rhizosphere: carbon trading at
 1525 the soil–root interface. *Plant Soil*.
- 1526 Kato, M., Ishikawa, S., Inagaki, K., Chiba, K., Hayashi, H., Yanagisawa, S. and Yoneyama,
 1527 T. 2010. Possible chemical forms of cadmium and varietal differences in cadmium
 1528 concentrations in the phloem sap of rice plants (*Oryza sativa* L.). *Soil Sci. Plant Nutr.*
 1529 **56**: 839-847.
- 1530 Kim, D.-Y., Bovet, L., Maeshima, M., Martinoia, E. and Lee, Y. 2007. The ABC transporter
 1531 AtPDR8 is a cadmium extrusion pump conferring heavy metal resistance. *Plant J.* **50**:
 1532 207-218.
- 1533 Kinraide, T. B. 1998. Three mechanisms for the calcium alleviation of mineral toxicities.
 1534 *Plant Physiol.* **118**: 513-520.

- 1535 Kobayashi, N. I., Tanoi, K., Hirose, A. and Nakanishi, T. M. 2013. Characterization of rapid
 1536 intervascular transport of cadmium in rice stem by radioisotope imaging. *J. Exp. Bot.*
 1537 **64**: 507-517.
- 1538 Koren'kov, V., Park, S., Cheng, N.-H., Sreevidya, C., Lachmansingh, J., Morris, J., Hirschi,
 1539 K. and Wagner, G. J. 2007. Enhanced Cd²⁺-selective root-tonoplast-transport in
 1540 tobaccos expressing *Arabidopsis* cation exchanger. *Planta* **225**: 403-411.
- 1541 Korenkov, V., King, B., Hirschi, K. and Wagner, G. J. 2009. Root-selective expression of
 1542 *AtCAX4* and *AtCAX2* results in reduced lamina cadmium in field-grown *Nicotiana*
 1543 *tabacum* L. *Plant Biotechnol. J.* **7**: 219-226.
- 1544 Korshunova, Y. O., Eide, D., Gregg Clark, W., Lou Guerinot, M. and Pakrasi, H. B. 1999.
 1545 The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad
 1546 substrate range. *Plant Mol. Biol.* **40**: 37-44.
- 1547 Kupper, H. and Andresen, E. 2016. Mechanisms of metal toxicity in plants. *Metallomics*.
- 1548 Küpper, H., Mijovilovitch, A., Meyer-Klaucke, W. and Kroneck, P. M. H. 2004. Tissue- and
 1549 age-dependant differences in the complexation of cadmium and zinc in the
 1550 cadmium/zinc hyperaccumulator *Thlaspi caerulescens* (Ganges ecotype) revealed by
 1551 X-ray absorption spectroscopy. *Plant Physiol.* **134**: 748-757.
- 1552 Kuramata, M., Masuya, S., Takahashi, Y., Kitagawa, E., Inoue, C., Ishikawa, S., Youssefi, S.
 1553 and Kusano, T. 2009. Novel cysteine-rich peptides from *Digitaria ciliaris* and *Oryza*
 1554 *sativa* enhance tolerance to cadmium by limiting its cellular accumulation. *Plant Cell*
 1555 *Physiol.* **50**: 107-117.
- 1556 Lackovic, K., Angove, M. J., Wells, J. D. and Johnson, B. B. 2004. Modeling the adsorption
 1557 of Cd(II) onto goethite in the presence of citric acid. *J. Colloid Interface Sci.* **269**: 37-
 1558 45.

- 1559 Lagerwerff, J. V. 1971. Uptake of cadmium, lead and zinc by radish from soil and air. *Soil*
 1560 *Sci.* **111**: 129-133.
- 1561 Lane, T. W., Saito, M. A., George, G. N., Pickering, I. J., Prince, R. C. and Morel, F. M. M.
 1562 2005. The first cadmium enzyme - carbonic anhydrase 2 from the marine diatom
 1563 *Thalassiosira weissflogii*. *SSRL Scienc High*.
- 1564 Lanquar, V., Lelièvre, F., Bolte, S., Hamès, C., Alcon, C., Neumann, D., Vansuyt, G., Curie,
 1565 C., Schröder, A. and Krämer, U. 2005. Mobilization of vacuolar iron by AtNRAMP3
 1566 and AtNRAMP4 is essential for seed germination on low iron. *EMBO J.* **24**: 4041-
 1567 4051.
- 1568 Lanquar, V., Ramos, M. S., Lelièvre, F., Barbier-Brygoo, H., Krieger-Liszkay, A., Krämer, U.
 1569 and Thomine, S. 2010. Export of vacuolar manganese by AtNRAMP3 and
 1570 AtNRAMP4 is required for optimal photosynthesis and growth under manganese
 1571 deficiency. *Plant Physiol.* **152**: 1986-1999.
- 1572 Laporte, M.-A., Denaix, L., Pagès, L., Sterckeman, T., Flénet, F., Dauguet, S. and Nguyen, C.
 1573 2013. Longitudinal variation in cadmium influx in intact first order lateral roots of
 1574 sunflower (*Helianthus annuus* L). *Plant Soil* **372**: 581-595.
- 1575 Laporte, M.-A., Sterckeman, T., Dauguet, S., Denaix, L. and Nguyen, C. 2015. Variability in
 1576 cadmium and zinc shoot concentration in 14 cultivars of sunflower (*Helianthus*
 1577 *annuus* L.) as related to metal uptake and partitioning. *Environ. Exp. Bot.* **109**: 45-53.
- 1578 Larsson Jönsson, E. H. and Asp, H. 2013. Effects of pH and nitrogen on cadmium uptake in
 1579 potato. *Biol. Plantarum* **57**: 788-792.
- 1580 Lee, S., Moon, J. S., Ko, T.-S., Petros, D., Goldsbrough, P. B. and Korban, S. S. 2003.
 1581 Overexpression of *Arabidopsis* phytochelatase synthase paradoxically leads to
 1582 hypersensitivity to cadmium stress. *Plant Physiol.* **131**: 656-663.

- 1583 Leitenmaier, B. and Küpper, H. 2011. Cadmium uptake and sequestration kinetics in
 1584 individual leaf cell protoplasts of the Cd/Zn hyperaccumulator *Thlaspi caerulescens*.
 1585 *Plant Cell Environ.* **34**: 208-219.
- 1586 Leopold, I., Günther, D., Schmidt, J. and Neumann, D. 1999. Phytochelatins and heavy metal
 1587 tolerance. *Phytochem.* **50**: 1323-1328.
- 1588 Leverrier, P., Montigny, C., Garrigos, M. and Champeil, P. 2007. Metal binding to ligands:
 1589 Cadmium complexes with glutathione revisited. *Anal. Biochem.* **371**: 215-228.
- 1590 Li, J.-Y., Fu, Y.-L., Pike, S. M., Bao, J., Tian, W., Zhang, Y., Chen, C.-Z., Zhang, Y., Li, H.-
 1591 M., Huang, J., Li, L.-G., Schroeder, J. I., Gassmann, W. and Gong, J.-M. 2010. The
 1592 *Arabidopsis* nitrate transporter NRT1.8 functions in nitrate removal from the xylem
 1593 sap and mediates cadmium tolerance. *Plant Cell* **22**: 1633-1646.
- 1594 Li, L., Liu, X., Peijnenburg, W. J. G. M., Zhao, J., Chen, X., Yu, J. and Wu, H. 2012a.
 1595 Pathways of cadmium fluxes in the root of the halophyte *Suaeda salsa*. *Ecotoxicol.*
 1596 *Environ. Safety* **75**: 1-7.
- 1597 Li, S. U. N., Yu, J., Zhu, M., Zhao, F. and Luan, S. 2012b. Cadmium impairs ion homeostasis
 1598 by altering K⁺ and Ca²⁺ channel activities in rice root hair cells. *Plant Cell Environ.*
 1599 **35**: 1998-2013.
- 1600 Li, T., Tao, Q., Shohag, M., Yang, X., Sparks, D. and Liang, Y. 2015. Root cell wall
 1601 polysaccharides are involved in cadmium hyperaccumulation in *Sedum alfredii*. *Plant*
 1602 *Soil* **389**: 387-399.
- 1603 Liang, J., Shohag, M. J. I., Yang, X., Tian, S., Zhang, Y., Feng, Y. and He, Z. 2014. Role of
 1604 sulfur assimilation pathway in cadmium hyperaccumulation by *Sedum alfredii* Hance.
 1605 *Ecotoxicol. Environ. Safety* **100**: 159-165.
- 1606 Liang, T., Ding, H., Wang, G., Kang, J., Pang, H. and Lv, J. 2016. Sulfur decreases cadmium
 1607 translocation and enhances cadmium tolerance by promoting sulfur assimilation and

- 1608 glutathione metabolism in *Brassica chinensis* L. *Ecotoxicol. Environ. Safety* **124**: 129-
1609 137.
- 1610 Liang, Y., Wong, J. W. C. and Wei, L. 2005. Silicon-mediated enhancement of cadmium
1611 tolerance in maize (*Zea mays* L.) grown in cadmium contaminated soil. *Chemosphere*
1612 **58**: 475-483.
- 1613 Lin, Y.-F. and Aarts, M. M. 2012. The molecular mechanism of zinc and cadmium stress
1614 response in plants. *Cell. Mol. Life Sci.* **69**: 3187-3206.
- 1615 Lin, Y.-F., Hassan, Z., Talukdar, S., Schat, H. and Aarts, M. G. M. 2016a. Expression of the
1616 ZNT1 zinc transporter from the metal hyperaccumulator *Noccaea caerulescens*
1617 confers enhanced zinc and cadmium tolerance and accumulation to *Arabidopsis*
1618 *thaliana*. *PLoS ONE* **11**: e0149750.
- 1619 Lin, Z., Schneider, A., Sterckeman, T. and Nguyen, C. 2016b. Ranking of mechanisms
1620 governing the phytoavailability of cadmium in agricultural soils using a mechanistic
1621 model. *Plant Soil* **399**: 89-107.
- 1622 Lindberg, S., Landberg, T. and Greger, M. 2007. Cadmium uptake and interaction with
1623 phytochelatins in wheat protoplasts. *Plant Physiol. Biochem.* **45**: 47-53.
- 1624 Liñero, O., Cornu, J.-Y., Candaudap, F., Pokrovsky, O. S., Bussière, S., Coriou, C., Humann-
1625 Guillemint, T., Robert, T., Thunot, S., de Diego, A. and Nguyen, C. 2016. Short-term
1626 partitioning of Cd recently taken up between sunflowers organs (*Helianthus annuus*)
1627 at flowering and grain filling stages: effect of plant transpiration and allometry. *Plant*
1628 *Soil* **408**: 163-181.
- 1629 Liñero, O., Cornu, J.-Y., de Diego, A., Bussière, S., Coriou, C., Thunot, S., Robert, T. and
1630 Nguyen, C. 2018. Source of Ca, Cd, Cu, Fe, K, Mg, Mn, Mo and Zn in grains of
1631 sunflower (*Helianthus annuus*) grown in nutrient solution: root uptake or
1632 remobilization from vegetative organs? *Plant Soil* **424**: 435-450.

- 1633 Liu, H., Zhang, J., Christie, P. and Zhang, F. 2008. Influence of iron plaque on uptake and
 1634 accumulation of Cd by rice (*Oryza sativa* L.) seedlings grown in soil. *Sc. Total*
 1635 *Environ.* **394**: 361-368.
- 1636 Liu, H., Zhao, H., Wu, L., Liu, A., Zhao, F.-J. and Xu, W. 2017. Heavy metal ATPase 3
 1637 (HMA3) confers cadmium hypertolerance on the cadmium/zinc hyperaccumulator
 1638 *Sedum plumbizincicola*. *New Phytol.* **215**: 687-698.
- 1639 Liu, H. J., Zhang, J. L. and Zhang, F. S. 2007. Role of iron plaque in Cd uptake by and
 1640 translocation within rice (*Oryza sativa* L.) seedlings grown in solution culture.
 1641 *Environ. Exp. Bot.* **59**: 314-320.
- 1642 Lofts, S. and Tipping, E. 1998. An assemblage model for cation binding by natural particulate
 1643 matter. *Geochim. Cosmochim. Acta* **62**: 2609-2625.
- 1644 Lombi, E., Tearall, K. L., Howarth, J. R., Zhao, F.-J., Hawkesford, M. J. and McGrath, S. P.
 1645 2002. Influence of iron status on cadmium and zinc uptake by different ecotypes of the
 1646 hyperaccumulator *Thlaspi caerulescens*. *Plant Physiol.* **128**: 1359-1367.
- 1647 Lombi, E., Zhao, F. J., McGrath, S. P., Young, S. D. and Sacchi, G. E. 2001. Physiological
 1648 evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi*
 1649 *caerulescens* ecotype. *New Phytol.* **149**: 53-60.
- 1650 Long, L., Persson, D. P., Duan, F., Jørgensen, K., Yuan, L., Schjoerring, J. K. and Pedas, P.
 1651 R. 2018. The iron-regulated transporter 1 plays an essential role in uptake,
 1652 translocation and grain-loading of manganese, but not iron, in barley. *New Phytol.*
 1653 **217**: 1640-1653.
- 1654 Lovy, L., Latt, D. and Sterckeman, T. 2013. Cadmium uptake and partitioning in the
 1655 hyperaccumulator *Noccaea caerulescens* exposed to constant Cd concentrations
 1656 throughout complete growth cycles. *Plant Soil*: 345–354.

- 1657 Lozano-Rodriguez, E., Hernández, L. E., Bonay, P. and Carpena-Ruiz, R. O. 1997.
 1658 Distribution of cadmium in shoot and root tissues of maize and pea plants.
 1659 Physiological disturbances. *J. Exp. Bot.* **48**: 123-128.
- 1660 Luo, B. F., Du, S. T., Lu, K. X., Liu, W. J., Lin, X. Y. and Jin, C. W. 2012. Iron uptake
 1661 system mediates nitrate-facilitated cadmium accumulation in tomato (*Solanum*
 1662 *lycopersicum*) plants. *J. Exp. Bot.* **63**: 3127-3136.
- 1663 Luo, J.-S., Huang, J., Zeng, D.-L., Peng, J.-S., Zhang, G.-B., Ma, H.-L., Guan, Y., Yi, H.-Y.,
 1664 Fu, Y.-L., Han, B., Lin, H.-X., Qian, Q. and Gong, J.-M. 2018. A defensin-like protein
 1665 drives cadmium efflux and allocation in rice. *Nature Communications* **9**: 645.
- 1666 Lux, A., Martinka, M., Vaculik, M. and White, P. J. 2011. Root responses to cadmium in the
 1667 rhizosphere: a review. *J. Exp. Bot.* **62**: 21-37.
- 1668 Maccaferri, M., Harris, N. S., Twardziok, S. O., Pasam, R. K., Gundlach, H., Spannagl, M.,
 1669 Ormanbekova, D., Lux, T., Prade, V. M., Milner, S. G., Himmelbach, A., Mascher,
 1670 M., Bagnaresi, P., Faccioli, P., Cozzi, P., Lauria, M., Lazzari, B., Stella, A., Manconi,
 1671 A., Gnocchi, M., Moscatelli, M., Avni, R., Deek, J., Biyiklioglu, S., Frascaroli, E.,
 1672 Corneti, S., Salvi, S., Sonnante, G., Desiderio, F., Marè, C., Crosatti, C., Mica, E.,
 1673 Özkan, H., Kilian, B., De Vita, P., Marone, D., Joukhadar, R., Mazzucotelli, E., Nigro,
 1674 D., Gadaleta, A., Chao, S., Faris, J. D., Melo, A. T. O., Pumphrey, M., Pecchioni, N.,
 1675 Milanesi, L., Wiebe, K., Ens, J., MacLachlan, R. P., Clarke, J. M., Sharpe, A. G., Koh,
 1676 C. S., Liang, K. Y. H., Taylor, G. J., Knox, R., Budak, H., Mastrangelo, A. M., Xu, S.
 1677 S., Stein, N., Hale, I., Distelfeld, A., Hayden, M. J., Tuberosa, R., Walkowiak, S.,
 1678 Mayer, K. F. X., Ceriotti, A., Pozniak, C. J. and Cattivelli, L. 2019. Durum wheat
 1679 genome highlights past domestication signatures and future improvement targets.
 1680 *Nature Genetics* **51**: 885-895.

- 1681 Mah, V. and Jalilehvand, F. 2010. Cadmium(II) complex formation with glutathione. *J. Biol.*
 1682 *Inorg. Chem.* **15**: 441-458.
- 1683 Marentes, E. and Rauser, W. E. 2007. Different proportions of cadmium occur as Cd-binding
 1684 phytochelatin complexes in plants. *Physiol. Plant.* **131**: 291-301.
- 1685 Martin-Garin, A., Van Cappellen, P. and Charlet, L. 2003. Aqueous cadmium uptake by
 1686 calcite: A stirred flow-through reactor study. *Geochim. Cosmochim. Acta* **67**: 2763-
 1687 2774.
- 1688 Maxted, A. P., Black, C. R., West, H. M., Crout, N. M. J., McGrath, S. P. and Young, S. D.
 1689 2007. Phytoextraction of cadmium and zinc from arable soils amended with sewage
 1690 sludge using *Thlaspi caerulescens*: Development of a predictive model. *Environ.*
 1691 *Pollut.* **150**: 363-372.
- 1692 McLaughlin, M. J., Andrew, S. J., Smart, M. K. and Smolders, E. 1998a. Effects of sulfate on
 1693 cadmium uptake by Swiss chard: I. Effects of complexation and calcium competition
 1694 in nutrient solutions. *Plant Soil* **202**: 211-216.
- 1695 McLaughlin, M. J., Lambrechts, R. M., Smolders, E. and Smart, M. K. 1998b. Effects of
 1696 sulfate on cadmium uptake by Swiss chard: II. Effects due to sulfate addition to soil.
 1697 *Plant Soil* **202**: 217-222.
- 1698 McLaughlin, M. J., Tiller, K. G. and Smart, M. K. 1997. Speciation of cadmium in soil
 1699 solutions of saline/sodic soils and relationship with cadmium concentrations in potato
 1700 tubers (*Solanum tuberosum* L.). *Aust. J. Soil Res.* **35**: 183-198.
- 1701 Meda, A. R., Scheuermann, E. B., Prechsl, U. E., Erenoglu, B., Schaaf, G., Hayen, H., Weber,
 1702 G. and von Wirén, N. 2007. Iron acquisition by phytosiderophores contributes to
 1703 cadmium tolerance. *Plant Physiol.* **143**: 1761-1773.
- 1704 Mendoza-Cozatl, D. G., Butko, E., Springer, F., Torpey, J. W., Komives, E. A., Kehr, J. and
 1705 Schroeder, J. I. 2008. Identification of high levels of phytochelatin, glutathione and

- 1706 cadmium in the phloem sap of *Brassica napus*. A role for thiol-peptides in the long-
 1707 distance transport of cadmium and the effect of cadmium on iron translocation. *Plant*
 1708 *J.* **54**: 249-259.
- 1709 Mengoni, A., Gonnelli, C., Hakvoort, H. W. J., Galardi, F., Bazzicalupo, M., Gabbrielli, R.
 1710 and Schat, H. 2003. Evolution of copper-tolerance and increased expression of a 2b-
 1711 type metallothionein gene in *Silene paradoxa* L. populations. *Plant Soil* **257**: 451-457.
- 1712 Meyer, C.-L., Juraniec, M., Huguet, S., Chaves-Rodriguez, E., Salis, P., Isaure, M.-P.,
 1713 Goormaghtigh, E. and Verbruggen, N. 2015. Intraspecific variability of cadmium
 1714 tolerance and accumulation, and cadmium-induced cell wall modifications in the metal
 1715 hyperaccumulator *Arabidopsis halleri*. *J. Exp. Bot.* **66**: 3215-3227.
- 1716 Migocka, M., Papierniak, A., Kosieradzka, A., Posyniak, E., Maciaszczyk-Dziubinska, E.,
 1717 Biskup, R., Garbiec, A. and Marchewka, T. 2015. Cucumber metal tolerance protein
 1718 CsMTP9 is a plasma membrane H⁺-coupled antiporter involved in the Mn²⁺ and Cd²⁺
 1719 efflux from root cells. *Plant J.* **84**: 1045-1058.
- 1720 Mills, R. F., Francini, A., Ferreira da Rocha, P. S. C., Baccarini, P. J., Aylett, M., Krijger, G.
 1721 C. and Williams, L. E. 2005. The plant P1B-type ATPase AtHMA4 transports Zn and
 1722 Cd and plays a role in detoxification of transition metals supplied at elevated levels.
 1723 *FEBS Lett.* **579**: 783-791.
- 1724 Mills, R. F., Krijger, G. C., Baccarini, P. J., Hall, J. L. and Williams, L. E. 2003. Functional
 1725 expression of AtHMA4, a P1B-type ATPase of the Zn/Co/Cd/Pb subclass. *Plant J.* **35**:
 1726 164-176.
- 1727 Milner, M. J., Craft, E., Yamaji, N., Koyama, E., Ma, J. F. and Kochian, L. V. 2012.
 1728 Characterization of the high affinity Zn transporter from *Noccaea caerulescens*,
 1729 NcZNT1, and dissection of its promoter for its role in Zn uptake and
 1730 hyperaccumulation. *New Phytol.* **195**: 113-123.

- 1731 Mirouze, M., Sels, J., Richard, O., Czernic, P., Loubet, S., Jacquier, A., François, I. E. J. A.,
 1732 Cammue, B. P. A., Lebrun, M., Berthomieu, P. and Marquès, L. 2006. A putative
 1733 novel role for plant defensins: a defensin from the zinc hyper-accumulating plant,
 1734 *Arabidopsis halleri*, confers zinc tolerance. *Plant J.* **47**: 329-342.
- 1735 Mishra, S., Mishra, A. and Küpper, H. 2017. Protein biochemistry and expression regulation
 1736 of cadmium/zinc pumping ATPases in the hyperaccumulator plants *Arabidopsis*
 1737 *halleri* and *Noccaea caerulescens*. *Frontiers Plant Sci.* **8**.
- 1738 Misono, M., Ochiai, E., Saito, Y. and Yoneda, Y. 1967. A new dual parameter scale for the
 1739 strength of lewis acids and bases with the evaluation of their softness. *Journal of*
 1740 *Inorganic and Nuclear Chemistry* **29**: 2685-2691.
- 1741 Miyadate, H., Adachi, S., Hiraizumi, A., Tezuka, K., Nakazawa, N., Kawamoto, T., Katou,
 1742 K., Kodama, I., Sakurai, K., Takahashi, H., Satoh-Nagasawa, N., Watanabe, A.,
 1743 Fujimura, T. and Akagi, H. 2011. OsHMA3, a P1B-type of ATPase affects root-to-
 1744 shoot cadmium translocation in rice by mediating efflux into vacuoles. *New Phytol.*
 1745 **189**: 190-199.
- 1746 Monsanto, A., Wang, Y. and Tang, C. 2010. Nitrate nutrition enhances zinc hyperaccumulation
 1747 in *Noccaea caerulescens* (Prayon). *Plant Soil* **336**: 391-404.
- 1748 Morel, M., Crouzet, J., Gravot, A., Auroy, P., Leonhardt, N., Vavasseur, A. and Richaud, P.
 1749 2009. AtHMA3, a P1B-ATPase allowing Cd/Zn/Co/Pb vacuolar storage in
 1750 *Arabidopsis*. *Plant Physiol.* **149**: 894-904.
- 1751 Moulis, J.-M. 2010. Cellular mechanisms of cadmium toxicity related to the homeostasis of
 1752 essential metals. *BioMetals* **23**: 877-896.
- 1753 Naidu, R. and Harter, R. D. 1998. Effect of different organic ligands on cadmium sorption by
 1754 extractability from soils. *Soil Sci. Soc. Am. J.* **62**: 644-650.

- 1755 Nakamura, S.-i., Suzui, N., Nagasaka, T., Komatsu, F., Ishioka, N. S., Ito-Tanabata, S.,
 1756 Kawachi, N., Rai, H., Hattori, H., Chino, M. and Fujimaki, S. 2013. Application of
 1757 glutathione to roots selectively inhibits cadmium transport from roots to shoots in
 1758 oilseed rape. *J. Exp. Bot.* **64**: 1073-1081.
- 1759 Nakanishi, H., Ogawa, I., Ishimaru, Y., Mori, S. and Nishizawa, N. K. 2006. Iron deficiency
 1760 enhances cadmium uptake and translocation mediated by the Fe²⁺ transporters OsIRT1
 1761 and OsIRT2 in rice. *Soil Sci. Plant Nutr.* **52**: 464-469.
- 1762 Nocito, F. F., Lancilli, C., Dendena, B., Lucchini, G. and Sacchi, G. A. 2011. Cadmium
 1763 retention in rice roots is influenced by cadmium availability, chelation and
 1764 translocation. *Plant Cell Environ.* **34**: 994-1008.
- 1765 Nocito, F. F., Pirovano, L., Cocucci, M. and Sacchi, G. A. 2002. Cadmium-induced sulfate
 1766 uptake in maize roots. *Plant Physiol.* **129**: 1872-1879.
- 1767 Nolan, A. L., McLaughlin, M. J. and Mason, S. D. 2003. Chemical speciation of Zn, Cd, Cu,
 1768 and Pb in pore waters of agricultural and contaminated soils using Donnan dialysis.
 1769 *Environ. Sci. Technol.* **37**: 90-98.
- 1770 Nowack, B., Schulin, R. and Robinson, B. H. 2006. Critical assessment of chelant-enhanced
 1771 metal phytoextraction. *Environ. Sci. Technol.* **40**: 5225-5232.
- 1772 Nwugo, C. C. and Huerta, A. J. 2008. Silicon-induced cadmium resistance in rice (*Oryza*
 1773 *sativa*). *J. Plant Nutr. Soil Sci.* **171**: 841-848.
- 1774 Ó Lochlainn, S., Bowen, H. C., Fray, R. G., Hammond, J. P., King, G. J., White, P. J.,
 1775 Graham, N. S. and Broadley, M. R. 2011. Tandem Quadruplication of HMA4 in the
 1776 Zinc (Zn) and Cadmium (Cd) Hyperaccumulator *Noccaea caerulescens*. *PLoS ONE* **6**:
 1777 e17814.
- 1778 Oomen, R. J. F. J., Wu, J., Lelièvre, F., Blanchet, S., Richaud, P., Barbier-Brygoo, H., Aarts,
 1779 M. G. M. and Thomine, S. 2009. Functional characterization of NRAMP3 and

- 1780 NRAMP4 from the metal hyperaccumulator *Thlaspi caerulescens*. *New Phytol.* **181**:
1781 637-650.
- 1782 Panfili, F., Schneider, A., Vives, A., Perrot, F., Hubert, P. and Pellerin, S. 2009. Cadmium
1783 uptake by durum wheat in presence of citrate. *Plant Soil* **316**: 299-309.
- 1784 Papoyan, A. and Kochian, L. V. 2004. Identification of *Thlaspi caerulescens* genes that may
1785 be involved in heavy metal hyperaccumulation and tolerance. Characterization of a
1786 novel heavy metal transporting ATPase. *Plant Physiol.* **136**: 3814-3823.
- 1787 Park, J., Song, W.-Y., Ko, D., Eom, Y., Hansen, T. H., Schiller, M., Lee, T. G., Martinoia, E.
1788 and Lee, Y. 2012. The phytochelatin transporters AtABCC1 and AtABCC2 mediate
1789 tolerance to cadmium and mercury. *Plant J.* **69**: 278-288.
- 1790 Pedas, P., Ytting, C. K., Fuglsang, A. T., Jahn, T. P., Schjoerring, J. K. and Husted, S. 2008.
1791 Manganese efficiency in barley: Identification and characterization of the metal ion
1792 transporter HvIRT1. *Plant Physiol.* **148**: 455-466.
- 1793 Pence, N. S., Larsen, P. B., Ebbs, S. D., Letham, D. L. D., Lasat, M. M., Garvin, D. F., Eide,
1794 D. and Kochian, L. V. 2000. The molecular physiology of heavy metal transport in the
1795 Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proc. Natl. Acad. Sci. U.S.A.* **97**:
1796 4956-4960.
- 1797 Peng, F., Wang, C., Zhu, J., Zeng, J., Kang, H., Fan, X., Sha, L., Zhang, H., Zhou, Y. and
1798 Wang, Y. 2018. Expression of TpNRAMP5, a metal transporter from Polish wheat
1799 (*Triticum polonicum* L.), enhances the accumulation of Cd, Co and Mn in transgenic
1800 Arabidopsis plants. *Planta* **247**: 1395-1406.
- 1801 Peng, J.-S., Ding, G., Meng, S., Yi, H.-Y. and Gong, J.-M. 2017a. Enhanced metal tolerance
1802 correlates with heterotypic variation in SpMTL, a metallothionein-like protein from
1803 the hyperaccumulator *Sedum plumbizincicola*. *Plant Cell Environ.* **40**: 1368-1378.

- 1804 Peng, J.-S., Wang, Y.-J., Ding, G., Ma, H.-L., Zhang, Y.-J. and Gong, J.-M. 2017b. A pivotal
 1805 role of cell wall in cadmium accumulation in the Crassulaceae hyperaccumulator
 1806 *Sedum plumbizincicola*. *Molecular Plant* **10**: 771-774.
- 1807 Perfus-Barbeoch, L., Leonhardt, N., Vavasseur, A. and Forestier, C. 2002. Heavy metal
 1808 toxicity: cadmium permeates through calcium channels and disturbs the plant water
 1809 status. *Plant J.* **32**: 539-548.
- 1810 Perriguet, J., Sterckeman, T. and Morel, J.-L. 2008. Effect of rhizosphere and plant-related
 1811 factors on the cadmium uptake by maize (*Zea mays* L.). *Environ. Exp. Bot.* **63**: 333-
 1812 341.
- 1813 Persson, D. P., Hansen, T. H., Holm, P. E., Schjoerring, J. K., Hansen, H. C. B., Nielsen, J.,
 1814 Cakmak, I. and Husted, S. 2006. Multi-elemental speciation analysis of barley
 1815 genotypes differing in tolerance to cadmium toxicity using SEC-ICP-MS and ESI-
 1816 TOF-MS. *J. Anal. Atom. Spectr.* **21**: 996-1005.
- 1817 Pineros, M. A., Shaff, J. E. and Kochian, L. V. 1998. Development, characterization, and
 1818 application of a cadmium-selective microelectrode for the measurement of cadmium
 1819 fluxes in roots of *Thlaspi* species and wheat. *Plant Physiol.* **116**: 1393-1401.
- 1820 Pinto, A. P., Simes, I. and Mota, A. M. 2008. Cadmium impact on root exudates of sorghum
 1821 and maize plants: A speciation study. *J. Plant Nutr.* **31**: 1746-1755.
- 1822 Pittman, J. K., Shigaki, T., Marshall, J. L., Morris, J. L., Cheng, N.-H. and Hirschi, K. D.
 1823 2004. Functional and regulatory analysis of the *Arabidopsis thaliana* CAX2 cation
 1824 transporter. *Plant Mol. Biol.* **56**: 959-971.
- 1825 Plaza, S., Tearall, K. L., Zhao, F.-J., Buchner, P., McGrath, S. P. and Hawkesford, M. J. 2007.
 1826 Expression and functional analysis of metal transporter genes in two contrasting
 1827 ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *J. Exp. Bot.* **58**: 1717-1728.

- 1828 Plaza, S., Weber, J., Pajonk, S., Thomas, J., Talke, I., Schellenberg, M., Pradervand, S., Burla,
 1829 B., Geisler, M., Martinoia, E. and Krämer, U. 2015. Wounding of *Arabidopsis halleri*
 1830 leaves enhances cadmium accumulation that acts as a defense against herbivory.
 1831 *BioMetals*: 1-8.
- 1832 Pongrac, P., Serra, T. S., Castillo-Michel, H., Vogel-Mikuš, K., Arčon, I., Kelemen, M.,
 1833 Jenčič, B., Kavčič, A., Villafort Carvalho, M. T. and Aarts, M. G. M. 2018. Cadmium
 1834 associates with oxalate in calcium oxalate crystals and competes with calcium for
 1835 translocation to stems in the cadmium bioindicator *Gomphrena claussenii*.
 1836 *Metallomics* **10**: 1576-1584.
- 1837 Pottier, M., Oomen, R., Picco, C., Giraudat, J., Scholz-Starke, J., Richaud, P., Carpaneto, A.
 1838 and Thomine, S. 2015. Identification of mutations allowing Natural Resistance
 1839 Associated Macrophage Proteins (NRAMP) to discriminate against cadmium. *Plant J.*
 1840 **83**: 625-637.
- 1841 Ptashnyk, M., Roose, T., Jones, D. L. and Kirk, G. J. D. 2011. Enhanced zinc uptake by rice
 1842 through phytosiderophore secretion: a modelling study. *Plant Cell Environ.* **34**: 2038-
 1843 2046.
- 1844 Puschenreiter, M., Gruber, B., Wenzel, W. W., Schindlegger, Y., Hann, S., Spangl, B.,
 1845 Schenkeveld, W. D. C., Kraemer, S. M. and Oburger, E. 2017. Phytosiderophore-
 1846 induced mobilization and uptake of Cd, Cu, Fe, Ni, Pb and Zn by wheat plants grown
 1847 on metal-enriched soils. *Environ. Exp. Bot.* **138**: 67-76.
- 1848 Qaswar, M., Hussain, S. and Rengel, Z. 2017. Zinc fertilisation increases grain zinc and
 1849 reduces grain lead and cadmium concentrations more in zinc-biofortified than standard
 1850 wheat cultivar. *Sc. Total Environ.* **605-606**: 454-460.

- 1851 Qin, Q., Li, X., Wu, H., Zhang, Y., Feng, Q. and Tai, P. 2013. Characterization of cadmium
1852 (^{108}Cd) distribution and accumulation in *Tagetes erecta* L. seedlings: Effect of split-
1853 root and of remove-xylem/phloem. *Chemosphere* **93**: 2284-2288.
- 1854 Qin, Q., Li, X., Zhuang, J., Weng, L., Liu, W. and Tai, P. 2015. Long-distance transport of
1855 cadmium from roots to leaves of *Solanum melongena*. *Ecotoxicol.* **24**: 2224-2232.
- 1856 Rahman, M. F., Ghosal, A., Alam, M. F. and Kabir, A. H. 2017. Remediation of cadmium
1857 toxicity in field peas (*Pisum sativum* L.) through exogenous silicon. *Ecotoxicol.*
1858 *Environ. Safety* **135**: 165-172.
- 1859 Rauser, W. E. 1995. Phytochelatins and related peptides. Structure, biosynthesis, and
1860 function. *Plant Physiol.* **109**: 1141-1149.
- 1861 Rauser, W. E. 2003. Phytochelatin-based complexes bind various amounts of cadmium in
1862 maize seedlings depending on the time of exposure, the concentration of cadmium and
1863 the tissue. *New Phytol.* **158**: 269-278.
- 1864 Redjala, T. 2009. Etude de l'absorption racinaire du cadmium afin d'améliorer la
1865 modélisation de son transfert vers la plante. Ecole Nationale d'Agronomie et des
1866 Industries Alimentaires. Nancy Université, Vandoeuvre-lès-Nancy.
- 1867 Redjala, T., Sterckeman, T. and Morel, J. L. 2009. Cadmium uptake by roots: Contribution of
1868 apoplast and of high- and low-affinity membrane transport systems. *Environ. Exp. Bot.*
1869 **67**: 235-242.
- 1870 Redjala, T., Sterckeman, T. and Morel, J. L. 2010a. Determination of the different
1871 components of cadmium short-term uptake by roots. *J. Plant Nutr. Soil Sci.* **173**: 935-
1872 945.
- 1873 Redjala, T., Sterckeman, T., Skiker, S. and Echevarria, G. 2010b. Contribution of apoplast
1874 and symplast to short term nickel uptake by maize and *Leptoplax emarginata* roots.
1875 *Environ. Exp. Bot.* **68**: 99-106.

- 1876 Redjala, T., Zelko, I., Sterckeman, T., Legué, V. and Lux, A. 2011. Relationship between root
1877 structure and root cadmium uptake in maize. *Environ. Exp. Bot.* **71**: 241-248.
- 1878 Ren, Y., Liu, Y., Chen, H., Li, G., Zhang, X. and Zhao, J. I. E. 2012. Type 4 metallothionein
1879 genes are involved in regulating Zn ion accumulation in late embryo and in controlling
1880 early seedling growth in *Arabidopsis*. *Plant Cell Environ.* **35**: 770-789.
- 1881 Ren, Z.-L., Tella, M., Bravin, M. N., Comans, R. N. J., Dai, J., Garnier, J.-M., Sivry, Y.,
1882 Doelsch, E., Straathof, A. and Benedetti, M. F. 2015a. Effect of dissolved organic
1883 matter composition on metal speciation in soil solutions. *Chem. Geol.* **398**: 61-69.
- 1884 Ren, Z. L., Sivry, Y., Dai, J., Tharaud, M., Cordier, L. and Benedetti, M. F. 2015b. Multi-
1885 element stable isotopic dilution and multi-surface modelling to assess the speciation
1886 and reactivity of cadmium and copper in soil. *Eur. J. Soil Sci.* **66**: 973-982.
- 1887 Rodriguez-Hernandez, M. C., Bonifas, I., Alfaro-De la Torre, M. C., Flores-Flores, J. L.,
1888 Bañuelos-Hernández, B. and Patiño-Rodríguez, O. 2015. Increased accumulation of
1889 cadmium and lead under Ca and Fe deficiency in *Typha latifolia*: A study of two pore
1890 channel (TPC1) gene responses. *Environ. Exp. Bot.* **115**: 38-48.
- 1891 Roosens, N., Leplae, R., Bernard, C. and Verbruggen, N. 2005. Variations in plant
1892 metallothioneins: the heavy metal hyperaccumulator *Thlaspi caerulescens* as a study
1893 case. *Planta* **222**: 716-729.
- 1894 Saifullah, Javed, H., Naeem, A., Rengel, Z. and Dahlawi, S. 2016. Timing of foliar Zn
1895 application plays a vital role in minimizing Cd accumulation in wheat. *Environ. Sci.*
1896 *Pollut. Res.* **23**: 16432-16439.
- 1897 Salt, D. E., Pickering, I. J., Prince, R. C., Gleba, D., Dushenkov, S., Smith, R. D. and Raskin,
1898 I. 1997. Metal accumulation by aquacultured seedlings of Indian mustard. *Environ.*
1899 *Sci. Technol.* **31**: 1636-1644.

- 1900 Salt, D. E., Prince, R. C., Pickering, I. J. and Raskin, I. 1995. Mechanisms of cadmium
1901 mobility and accumulation in Indian Mustard. *Plant Physiol.* **109**: 1427-1433.
- 1902 Salt, D. E. and Wagner, G. J. 1993. Cadmium transport across tonoplast of vesicles from oat
1903 roots. Evidence for a $\text{Cd}^{2+}/\text{H}^{+}$ antiport activity. *J. Biol. Chem.* **268**: 12297-12302.
- 1904 Sarret, G., Vangronsveld, J., Manceau, A., Musso, M., D'Haen, J., Menthonnex, J.-J. and
1905 Hazemann, J.-L. 2001. Accumulation forms of Zn and Pb in *Phaseolus vulgaris* in the
1906 presence and absence of EDTA. *Environ. Sci. Technol.* **35**: 2854-2859.
- 1907 Sasaki, A., Yamaji, N., Yokosho, K. and Ma, J. F. 2012. Nramp5 is a major transporter
1908 responsible for manganese and cadmium uptake in rice. *Plant Cell* **24**: 2155-2167.
- 1909 Satoh-Nagasawa, N., Mori, M., Nakazawa, N., Kawamoto, T., Nagato, Y., Sakurai, K.,
1910 Takahashi, H., Watanabe, A. and Akagi, H. 2012. Mutations in rice (*Oryza sativa*)
1911 Heavy Metal ATPase 2 (OsHMA2) restrict the translocation of zinc and cadmium.
1912 *Plant Cell Physiol.* **53**: 213-224.
- 1913 Sauvé, S., Hendershot, W. and Allen, H. E. 2000a. Solid-solution partitioning of metals in
1914 contaminated soils: Dependence on pH, total metal burden, and organic matter.
1915 *Environ. Sci. Technol.* **34**: 1125-1131.
- 1916 Sauvé, S., Norvell, W. A., McBride, M. and Hendershot, W. 2000b. Speciation and
1917 complexation of cadmium in extracted soil solutions. *Environ. Sci. Technol.* **34**: 291-
1918 296.
- 1919 Schaider, L., Parker, D. and Sedlak, D. 2006. Uptake of EDTA-complexed Pb, Cd and Fe by
1920 solution- and sand-cultured *Brassica juncea*. *Plant Soil* **286**: 377-391.
- 1921 Schat, H., Llugany, M., Vooijs, R., Hartley-Whitaker, J. and Bleeker, P. M. 2002. The role of
1922 phytochelatins in constitutive and adaptive heavy metal tolerances in
1923 hyperaccumulator and non-hyperaccumulator metallophytes. *J. Exp. Bot.* **53**: 2381-
1924 2392.

- 1925 Schneider, A. 2008. An exchange method to investigate the kinetics of Cd complexation in
1926 soil solutions. *Environ. Sci. Technol.* **42**: 4076-4082.
- 1927 Schneider, A. and Nguyen, C. 2011. Use of an exchange method to estimate the association
1928 and dissociation rate constants of cadmium complexes formed with low-molecular-
1929 weight organic acids commonly exuded by plant roots. *J. Environ. Quality* **40**: 1857-
1930 1862.
- 1931 Schneider, T., Schellenberg, M., Meyer, S., Keller, F., Gehrig, P., Riedel, K., Lee, Y., Eberl,
1932 L. and Martinoia, E. 2009. Quantitative detection of changes in the leaf-mesophyll
1933 tonoplast proteome in dependency of a cadmium exposure of barley (*Hordeum*
1934 *vulgare* L.) plants. *Proteomics* **9**: 2668-2677.
- 1935 Sebastian, A. and Prasad, M. N. V. 2016. Iron plaque decreases cadmium accumulation in
1936 *Oryza sativa* L. and serves as a source of iron. *Plant Biol.* **18**: 1008-1015.
- 1937 Senden, M., van der Meer, A., Verburg, T. and Wolterbeek, H. 1995. Citric acid in tomato
1938 plant roots and its effect on cadmium uptake and distribution. *Plant Soil* **171**: 333-339.
- 1939 Shabala, S. 2007. Transport from root to shoot. In: Yeo, A. R. and Flowers, T. J. (eds) *Plant*
1940 *Solute Transport*. Blackwell Publishing, Oxford.
- 1941 Shao, J. F., Che, J., Yamaji, N., Shen, R. F. and Ma, J. F. 2017. Silicon reduces cadmium
1942 accumulation by suppressing expression of transporter genes involved in cadmium
1943 uptake and translocation in rice. *J. Exp. Bot.* **68**: 5641-5651.
- 1944 Shao, J. F., Xia, J., Yamaji, N., Shen, R. F. and Ma, J. F. 2018. Effective reduction of
1945 cadmium accumulation in rice grain by expressing OsHMA3 under the control of the
1946 OsHMA2 promoter. *J. Exp. Bot.* **69**: 2743-2752.
- 1947 Shen, G.-m., Du, Q.-z. and Wang, J.-x. 2012. Involvement of plasma membrane $\text{Ca}^{2+}/\text{H}^{+}$
1948 antiporter in Cd^{2+} tolerance. *Rice Science* **19**: 161-165.

- 1949 Shenker, M., Fan, T. W.-M. and Crowley, D. E. 2001. Phytosiderophores influence on
 1950 cadmium mobilization and uptake by wheat and barley plants. *J. Environ. Quality* **30**:
 1951 2091-2098.
- 1952 Shigaki, T. and Hirschi, K. D. 2006. Diverse functions and molecular properties emerging for
 1953 CAX Cation/H⁺ exchangers in plants. *Plant Biol.* **8**: 419-429.
- 1954 Shojima, S., Nishizawa, N.-K., Fushiya, S., Nozoe, S., Irifune, T. and Mori, S. 1990.
 1955 Biosynthesis of phytosiderophores. In vitro biosynthesis of 2'-deoxymugineic acid
 1956 from L-methionine and nicotianamine. *Plant Physiol.* **93**: 1497-1503.
- 1957 Six, L. and Smolders, E. 2014. Future trends in soil cadmium concentration under current
 1958 cadmium fluxes to European agricultural soils. *Sc. Total Environ.* **485–486**: 319-328.
- 1959 Smolders, E., Brans, K., Földi, A. and Merckx, R. 1999. Cadmium fixation in soils measured
 1960 by isotopic dilution. *Soil Sci. Soc. Am. J.* **63**: 78-85.
- 1961 Smolders, E. and McLaughlin, M. J. 1996a. Chloride increases cadmium uptake in swiss
 1962 chard in a resin-buffered nutrient solution. *Soil Sci. Soc. Am. J.* **60**: 1443-1447.
- 1963 Smolders, E. and McLaughlin, M. J. 1996b. Effect of Cl on Cd uptake by Swiss chard in
 1964 nutrient solutions. *Plant Soil* **179**: 57-64.
- 1965 Song, W.-Y., Choi, K. S., Kim, D. Y., Geisler, M., Park, J., Vincenzetti, V., Schellenberg, M.,
 1966 Kim, S. H., Lim, Y. P., Noh, E. W., Lee, Y. and Martinoia, E. 2010. *Arabidopsis*
 1967 PCR2 is a zinc exporter involved in both zinc extrusion and long-distance zinc
 1968 transport. *Plant Cell* **22**: 2237-2252.
- 1969 Song, W.-Y., Martinoia, E., Lee, J., Kim, D., Kim, D.-Y., Vogt, E., Shim, D., Choi, K. S.,
 1970 Hwang, I. and Lee, Y. 2004. A novel family of cys-rich membrane proteins mediates
 1971 cadmium resistance in *Arabidopsis*. *Plant Physiol.* **135**: 1027-1039.
- 1972 Song, W.-Y., Mendoza-CÓZatl, D. G., Lee, Y., Schroeder, J. I., Ahn, S.-N., Lee, H.-S.,
 1973 Wicker, T. and Martinoia, E. 2014. Phytochelatin–metal(loid) transport into vacuoles

- 1974 shows different substrate preferences in barley and *Arabidopsis*. *Plant Cell Environ.*
 1975 **37**: 1192-1201.
- 1976 Sposito, G. 1989. The chemistry of soils. Oxford University Press, Oxford.
- 1977 Sterckeman, T., Carignan, J., Srayeddin, I., Baize, D. and Cloquet, C. 2009. Availability of
 1978 soil cadmium using stable and radioactive isotope dilution. *Geoderma* **153**: 372-378.
- 1979 Sterckeman, T., Cazes, Y., Gonneau, C. and Sirguey, C. 2017. Phenotyping 60 populations of
 1980 *Noccaea caerulea* provides a broader knowledge of variation in traits of interest
 1981 for phytoextraction. *Plant Soil* **418**: 523-540.
- 1982 Sterckeman, T., Duquene, L., Perriguet, J. and Morel, J. L. 2005. Quantifying the effect of
 1983 rhizosphere processes on the availability of soil cadmium and zinc. *Plant Soil* **276**:
 1984 335-345.
- 1985 Sterckeman, T., Gossiaux, L., Guimont, S., Sirguey, C. and Lin, Z. 2018. Cadmium mass
 1986 balance in French soils under annual crops: Scenarios for the next century. *Sc. Total*
 1987 *Environ.* **639**: 1440-1452.
- 1988 Sterckeman, T. and Puschenreiter, M. 2018. Phytoextraction of Cadmium: Feasibility in Field
 1989 Applications and Potential Use of Harvested Biomass. In: Van der Ent, A., Echevarria,
 1990 G., Baker, A. J. M. and Morel, J. L. (eds) Agromining: Farming for Metals: Extracting
 1991 Unconventional Resources Using Plants. Springer International Publishing, Cham.
- 1992 Sterckeman, T., Redjala, T. and Morel, J. L. 2011. Influence of exposure solution composition
 1993 and of plant cadmium content on root cadmium short-term uptake. *Environ. Exp. Bot.*
 1994 **74**: 131-139.
- 1995 Sui, F.-Q., Chang, J.-D., Tang, Z., Liu, W.-J., Huang, X.-Y. and Zhao, F.-J. 2018. Nramp5
 1996 expression and functionality likely explain higher cadmium uptake in rice than in
 1997 wheat and maize. *Plant Soil* **433**: 377-389.

- 1998 Sui, F., Zhao, D., Zhu, H., Gong, Y., Tang, Z., Huang, X.-Y., Zhang, G. and Zhao, F.-J. 2019.
- 1999 Map-based cloning of a new total loss-of-function allele of OsHMA3 causes high
- 2000 cadmium accumulation in rice grain. *J. Exp. Bot.* **70**: 2857-2871.
- 2001 Sun, C., Yang, M., Li, Y., Tian, J., Zhang, Y., Liang, L., Liu, Z., Chen, K., Li, Y., Lv, K. and
- 2002 Lian, X. 2019. Comprehensive analysis of variation of cadmium accumulation in rice
- 2003 and detection of a new weak allele of OsHMA3. *J. Exp. Bot.* **70**: 6389-6400.
- 2004 Sun, Q., Ye, Z. H., Wang, X. R. and Wong, M. H. 2007a. Cadmium hyperaccumulation leads
- 2005 to an increase of glutathione rather than phytochelatins in the cadmium
- 2006 hyperaccumulator *Sedum alfredii*. *J. Plant Physiol.* **164**: 1489-1498.
- 2007 Sun, R.-L., Zhou, Q.-X., Sun, F.-H. and Jin, C.-X. 2007b. Antioxidative defense and
- 2008 proline/phytochelatin accumulation in a newly discovered Cd-hyperaccumulator,
- 2009 *Solanum nigrum* L. *Environ. Exp. Bot.* **60**: 468-476.
- 2010 Takahashi, R., Ishimaru, Y., Senoura, T., Shimo, H., Ishikawa, S., Arao, T., Nakanishi, H. and
- 2011 Nishizawa, N. K. 2011. The OsNRAMP1 iron transporter is involved in Cd
- 2012 accumulation in rice. *J. Exp. Bot.* **62**: 4843-4850.
- 2013 Takahashi, R., Ishimaru, Y., Shimo, H., Ogo, Y., Senoura, T., Nishizawa, N. K. and
- 2014 Nakanishi, H. 2012. The OsHMA2 transporter is involved in root-to-shoot
- 2015 translocation of Zn and Cd in rice. *Plant Cell Environ.* **35**: 1948-1957.
- 2016 Tanaka, K., Fujimaki, S., Fujiwara, T., Yoneyama, T. and Hayashi, H. 2007. Quantitative
- 2017 estimation of the contribution of the phloem in cadmium transport to grains in rice
- 2018 plants (*Oryza sativa* L.). *Soil Sci. Plant Nutr.* **53**: 72-77.
- 2019 Tanaka, N., Nishida, S., Kamiya, T. and Fujiwara, T. 2016. Large-scale profiling of brown
- 2020 rice ionome in an ethyl methanesulphonate-mutagenized hitomebore population and
- 2021 identification of high- and low-cadmium lines. *Plant Soil* **407**: 109-117.

- 2022 Tang, L., Mao, B., Li, Y., Lv, Q., Zhang, L., Chen, C., He, H., Wang, W., Zeng, X., Shao, Y.,
2023 Pan, Y., Hu, Y., Peng, Y., Fu, X., Li, H., Xia, S. and Zhao, B. 2017. Knockout of
2024 OsNramp5 using the CRISPR/Cas9 system produces low Cd-accumulating indica rice
2025 without compromising yield. *Sci. Rep.* **7**: 14438.
- 2026 Tao, Q., Hou, D., Yang, X. and Li, T. 2015. Oxalate secretion from the root apex of *Sedum*
2027 *alfredii* contributes to hyperaccumulation of Cd. *Plant Soil* **398**: 139-152.
- 2028 Tao, Q., Jupa, R., Luo, J., Lux, A., Kováč, J., Wen, Y., Zhou, Y., Jan, J., Liang, Y. and Li, T.
2029 2017. The apoplasmic pathway via the root apex and lateral roots contributes to Cd
2030 hyperaccumulation in the hyperaccumulator *Sedum alfredii*. *J. Exp. Bot.* **68**: 739-751.
- 2031 Thomine, S., Lelièvre, F., Debarbieux, E., Schroeder, J. I. and Barbier-Brygoo, H. 2003.
2032 AtNRAMP3, a multispecific vacuolar metal transporter involved in plant responses to
2033 iron deficiency. *Plant J.* **34**: 685-695.
- 2034 Thomine, S., Wang, R., Ward, J. M., Crawford, N. M. and Schroeder, J. I. 2000. Cadmium
2035 and iron transport by members of a plant metal transporter family in *Arabidopsis* with
2036 homology to Nramp genes. *Proc. Natl. Acad. Sci. U.S.A.* **97**: 4991-4996.
- 2037 Tian, S., Lu, L., Labavitch, J., Yang, X., He, Z., Hu, H., Sarangi, R., Newville, M.,
2038 Commisso, J. and Brown, P. 2011. Cellular sequestration of cadmium in the
2039 hyperaccumulator plant species *Sedum alfredii*. *Plant Physiol.* **157**: 1914-1925.
- 2040 Tian, S., Xie, R., Wang, H., Hu, Y., Ge, J., Liao, X., Gao, X., Brown, P., Lin, X. and Lu, L.
2041 2016. Calcium deficiency triggers phloem remobilization of cadmium in a
2042 hyperaccumulating species. *Plant Physiol.* **172**: 2300-2313.
- 2043 Tian, S., Xie, R., Wang, H., Hu, Y., Hou, D., Liao, X., Brown, P. H., Yang, H., Lin, X.,
2044 Labavitch, J. M. and Lu, L. 2017. Uptake, sequestration and tolerance of cadmium at
2045 cellular levels in the hyperaccumulator plant species *Sedum alfredii*. *J. Exp. Bot.* **68**:
2046 2387-2398.

- 2047 Tipping, E. 1998. Humic ion-binding model VI: An improved description of the interactions
2048 of protons and metal ions with humic substances. *Aquatic Geochem.* **4**: 3-47.
- 2049 Tommasini, R., Vogt, E., Fromenteau, M., Hörtensteiner, S., Matile, P., Amrhein, N. and
2050 Martinoia, E. 1998. An ABC-transporter of *Arabidopsis thaliana* has both glutathione-
2051 conjugate and chlorophyll catabolite transport activity. *Plant J.* **13**: 773-780.
- 2052 Tye, A. M., Young, S. D., Crout, N. M. J., Zhang, H., Preston, S., Barbosa-Jefferson, V. L.,
2053 Davison, W., McGrath, S. P., Paton, G. I., Kilham, K. and Resende, L. 2003.
2054 Predicting the activity of Cd^{2+} and Zn^{2+} in soil pore water from the radio-labile metal
2055 fraction. *Geochim. Cosmochim. Acta* **67**: 375-385.
- 2056 Ueno, D., Iwashita, T., Zhao, F.-J. and Ma, J. F. 2008. Characterization of Cd translocation
2057 and identification of the Cd form in xylem sap of the Cd-hyperaccumulator
2058 *Arabidopsis halleri*. *Plant Cell Physiol.* **49**: 540-548.
- 2059 Ueno, D., Koyama, E., Yamaji, N. and Ma, J. F. 2011a. Physiological, genetic, and molecular
2060 characterization of a high-Cd-accumulating rice cultivar, Jarjan. *J. Exp. Bot.* **62**: 2265-
2061 2272.
- 2062 Ueno, D., Ma, J. F., Iwashita, T., Zhao, F.-J. and McGrath, S. P. 2005. Identification of the
2063 form of Cd in the leaves of a superior Cd-accumulating ecotype of *Thlaspi*
2064 *caerulescens* using ^{113}Cd -NMR. *Planta* **221**: 928-936.
- 2065 Ueno, D., Milner, M. J., Yamaji, N., Yokosho, K., Koyama, E., Clemencia Zambrano, M.,
2066 Kaskie, M., Ebbs, S., Kochian, L. V. and Ma, J. F. 2011b. Elevated expression of
2067 TcHMA3 plays a key role in the extreme Cd tolerance in a Cd-hyperaccumulating
2068 ecotype of *Thlaspi caerulescens*. *Plant J.* **66**: 852-862.
- 2069 Ueno, D., Sasaki, A., Yamaji, N., Miyaji, T., Fujii, Y., Takemoto, Y., Moriyama, S., Che, J.,
2070 Moriyama, Y., Iwasaki, K. and Ma, J. F. 2015. A polarly localized transporter for
2071 efficient manganese uptake in rice. *Nature Plants* **1**: 15170.

- 2072 Ueno, D., Yamaji, N., Kono, I., Huang, C. F., Ando, T., Yano, M. and Ma, J. F. 2010. Gene
2073 limiting cadmium accumulation in rice. *Proc. Natl. Acad. Sci.* **107**: 16500-16505.
- 2074 Uraguchi, S., Kamiya, T., Clemens, S. and Fujiwara, T. 2014. Characterization of OsLCT1, a
2075 cadmium transporter from indica rice (*Oryza sativa*). *Physiol. Plant.* **151**: 339-347.
- 2076 Uraguchi, S., Kamiya, T., Sakamoto, T., Kasai, K., Sato, Y., Nagamura, Y., Yoshida, A.,
2077 Kyozuka, J., Ishikawa, S. and Fujiwara, T. 2011. Low-affinity cation transporter
2078 (OsLCT1) regulates cadmium transport into rice grains. *Proc. Natl. Acad. Sci.* **108**:
2079 20959-20964.
- 2080 Uraguchi, S., Kiyono, M., Sakamoto, T., Watanabe, I. and Kuno, K. 2009a. Contributions of
2081 apoplasmic cadmium accumulation, antioxidative enzymes and induction of
2082 phytochelatins in cadmium tolerance of the cadmium-accumulating cultivar of black
2083 oat (*Avena strigosa* Schreb.). *Planta* **230**: 267-276.
- 2084 Uraguchi, S., Mori, S., Kuramata, M., Kawasaki, A., Arao, T. and Ishikawa, S. 2009b. Root-
2085 to-shoot Cd translocation via the xylem is the major process determining shoot and
2086 grain cadmium accumulation in rice. *J. Exp. Bot.* **60**: 2677-2688.
- 2087 Vaculík, M., Landberg, T., Greger, M., Luxová, M., Stoláriková, M. and Lux, A. 2012.
2088 Silicon modifies root anatomy, and uptake and subcellular distribution of cadmium in
2089 young maize plants. *Ann. Bot.* **110**: 433-443.
- 2090 Vaculík, M., Lux, A., Luxová, M., Tanimoto, E. and Lichtscheidl, I. 2009. Silicon mitigates
2091 cadmium inhibitory effects in young maize plants. *Environ. Exp. Bot.* **67**: 52-58.
- 2092 Van Belleghem, F., Cuypers, A., Semane, B., Smeets, K., Vangronsveld, J., d'Haen, J. and
2093 Valcke, R. 2007. Subcellular localization of cadmium in roots and leaves of
2094 *Arabidopsis thaliana*. *New Phytol.* **173**: 495-508.
- 2095 van de Mortel, J. E., Almar Villanueva, L., Schat, H., Kwekkeboom, J., Coughlan, S.,
2096 Moerland, P. D., Ver Loren van Themaat, E., Koornneef, M. and Aarts, M. G. M.

- 2097 2006. Large expression differences in genes for iron and zinc homeostasis, stress
 2098 response, and lignin biosynthesis distinguish roots of *Arabidopsis thaliana* and the
 2099 related metal hyperaccumulator *Thlaspi caerulescens*. *Plant Physiol.* **142**: 1127-1147.
- 2100 van der Ent, A., Baker, A. J. M., Reeves, R. D., Pollard, A. J. and Schat, H. 2013.
 2101 Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. *Plant Soil*
 2102 **362**: 319-334.
- 2103 Van der Vliet, L., Peterson, C. and Hale, B. 2007. Cd accumulation in roots and shoots of
 2104 durum wheat: the roles of transpiration rate and apoplastic bypass. *J. Exp. Bot.* **58**:
 2105 2939-2947.
- 2106 van Hoof, N. A. L. M., Hassinen, V. H., Hakvoort, H. W. J., Ballintijn, K. F., Schat, H.,
 2107 Verkleij, J. A. C., Ernst, W. H. O., Karenlampi, S. O. and Tervahauta, A. I. 2001.
 2108 Enhanced copper tolerance in *Silene vulgaris* (Moench) Garcke populations from
 2109 copper mines is associated with increased transcript levels of a 2b-type
 2110 metallothionein gene. *Plant Physiol.* **126**: 1519-1526.
- 2111 Vatamaniuk, O. K., Mari, S., Lu, Y.-P. and Rea, P. A. 1999. AtPCS1, a phytochelatin
 2112 synthase from *Arabidopsis*: Isolation and *in vitro* reconstitution. *Proc. Natl. Acad. Sci.*
 2113 **96**: 7110-7115.
- 2114 Vatamaniuk, O. K., Mari, S., Lu, Y.-P. and Rea, P. A. 2000. Mechanism of heavy metal ion
 2115 activation of phytochelatin (PC) synthase blocked thiols are sufficient for PC
 2116 synthase-catalyzed transpeptidation of glutathione and related thiol peptides. *J. Biol.*
 2117 *Chem.* **275**: 31451-31459.
- 2118 Vazquez, S., Goldsbrough, P. and Carpena, R. O. 2006. Assessing the relative contributions
 2119 of phytochelatin and the cell wall to cadmium resistance in white lupin. *Physiol.*
 2120 *Plant.* **128**: 487-495.

- 2121 Verbruggen, N., Hermans, C. and Schat, H. 2009a. Mechanisms to cope with arsenic or
2122 cadmium excess in plants. *Curr. Opin. Plant Biol.* **12**: 364-372.
- 2123 Verbruggen, N., Hermans, C. and Schat, H. 2009b. Molecular mechanisms of metal
2124 hyperaccumulation in plants. *New Phytol.* **181**: 759-776.
- 2125 Verbruggen, N., Juraniec, M., Baliardini, C. and Meyer, C.-L. 2013. Tolerance to cadmium in
2126 plants: the special case of hyperaccumulators. *BioMetals* **26**: 633-638.
- 2127 Verret, F., Gravot, A., Auroy, P., Leonhardt, N., David, P., Nussaume, L., Vavasseur, A. and
2128 Richaud, P. 2004. Overexpression of AtHMA4 enhances root-to-shoot translocation of
2129 zinc and cadmium and plant metal tolerance. *FEBS Lett.* **576**: 306-312.
- 2130 Vert, G., Grotz, N., Dédaldéchamp, F., Gaymard, F., Guerinot, M. L., Briat, J.-F. and Curie,
2131 C. 2002. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and
2132 for plant growth. *Plant Cell* **14**: 1223-1233.
- 2133 Vieira da Cunha, K. P., Araújo do Nascimento, C. W. and da Silva, A. J. 2008. Silicon
2134 alleviates the toxicity of cadmium and zinc for maize (*Zea mays* L.) grown on a
2135 contaminated soil. *J. Plant Nutr. Soil Sci.* **171**: 849-853.
- 2136 Vogel-Mikuš, K., Arčon, I. and Kodre, A. 2010. Complexation of cadmium in seeds and
2137 vegetative tissues of the cadmium hyperaccumulator *Thlaspi praecox* as studied by X-
2138 ray absorption spectroscopy. *Plant Soil* **331**: 439-451.
- 2139 Vogel-Mikus, K., Pongrac, P., Kump, P., Necemer, M., Simcic, J., Pelicon, P., Budnar, M.,
2140 Povh, B. and Regvar, M. 2007. Localisation and quantification of elements within
2141 seeds of Cd/Zn hyperaccumulator *Thlaspi praecox* by micro-PIXE. *Environ. Pollut.*
2142 **147**: 50-59.
- 2143 Vogel-Mikus, K., Regvar, M., Mesjasz-Przybylowicz, J., Przybylowicz, W. J., Simcic, J.,
2144 Pelicon, P. and Budnar, M. 2008. Spatial distribution of cadmium in leaves of metal
2145 hyperaccumulating *Thlaspi praecox* using micro-PIXE. *New Phytol.* **179**: 712-721.

- 2146 Vögeli-Lange, R. and Wagner, G. J. 1990. Subcellular localization of cadmium and cadmium-
 2147 binding peptides in tobacco leaves. Implication of a transport function for cadmium-
 2148 binding peptides. *Plant Physiol.* **92**: 1086-1093.
- 2149 Vollenweider, P., Cosio, C., Gunthardt-Goerg, M. S. and Keller, C. 2006. Localization and
 2150 effects of cadmium in leaves of a cadmium-tolerant willow (*Salix viminalis* L.): Part II
 2151 Microlocalization and cellular effects of cadmium. *Environ. Exp. Bot.* **58**: 25-40.
- 2152 von Wirén, N., Marschner, H. and Römheld, V. 1996. Roots of iron-efficient maize also
 2153 absorb phytosiderophore-chelated zinc. *Plant Physiol.* **111**: 1119-1125.
- 2154 Wang, J., Su, L., Yang, J., Yuan, J., Yin, A., Qiu, Q., Zhang, K. and Yang, Z. 2015a.
 2155 Comparisons of cadmium subcellular distribution and chemical forms between low-Cd
 2156 and high-Cd accumulation genotypes of watercress (*Nasturtium officinale* L. R. Br.).
 2157 *Plant Soil* **396**: 325-337.
- 2158 Wang, P., De Schamphelaere, K. A. C., Kopittke, P. M., Zhou, D.-M., Peijnenburg, W. J. G.
 2159 M. and Lock, K. 2012. Development of an electrostatic model predicting copper
 2160 toxicity to plants. *J. Exp. Bot.* **63**: 659-668.
- 2161 Wang, P., Deng, X., Huang, Y., Fang, X., Zhang, J., Wan, H. and Yang, C. 2015b.
 2162 Comparison of subcellular distribution and chemical forms of cadmium among four
 2163 soybean cultivars at young seedlings. *Environ. Sci. Pollut. Res.* **22**: 19584-19595.
- 2164 Wang, P., Kinraide, T. B., Zhou, D., Kopittke, P. M. and Peijnenburg, W. J. G. M. 2011.
 2165 Plasma membrane surface potential: dual effects upon ion uptake and toxicity. *Plant*
 2166 *Physiol.* **155**: 808-820.
- 2167 Wang, P., Zhou, D., Luo, X. and Li, L. 2009. Effects of Zn-complexes on zinc uptake by
 2168 wheat (*Triticum aestivum*) roots: a comprehensive consideration of physical, chemical
 2169 and biological processes on biouptake. *Plant Soil* **316**: 177-192.

- 2170 Wang, S., Wang, F. and Gao, S. 2015c. Foliar application with nano-silicon alleviates Cd
2171 toxicity in rice seedlings. *Environ. Sci. Pollut. Res.* **22**: 2837-2845.
- 2172 Wang, Y., Wang, C., Liu, Y., Yu, K. and Zhou, Y. 2018. GmHMA3 sequesters Cd to the root
2173 endoplasmic reticulum to limit translocation to the stems in soybean. *Plant Sci.* **270**:
2174 23-29.
- 2175 Weggler, K., McLaughlin, M. J. and Graham, R. D. 2004. Effect of chloride in soil solution
2176 on the plant availability of biosolid-borne cadmium. *J. Environ. Quality* **33**: 496-504.
- 2177 Wei, S., Anders, I. and Feller, U. 2014. Selective uptake, distribution, and redistribution of
2178 ¹⁰⁹Cd, ⁵⁷Co, ⁶⁵Zn, ⁶³Ni, and ¹³⁴Cs via xylem and phloem in the heavy metal
2179 hyperaccumulator *Solanum nigrum* L. *Environ. Sci. Pollut. Res.* **21**: 7624-7630.
- 2180 Wei, S., Zhou, Q. and Koval, P. V. 2006. Flowering stage characteristics of cadmium
2181 hyperaccumulator *Solanum nigrum* L. and their significance to phytoremediation. *Sc.*
2182 *Total Environ.* **369**: 441-446.
- 2183 Wei, Z. G., Wong, J. W. C., Zhao, H. Y., Zhang, H. J., Li, H. X. and Hu, F. 2007. Separation
2184 and determination of heavy metals associated with low molecular weight chelators in
2185 xylem saps of Indian mustard (*Brassica juncea*) by size exclusion chromatography and
2186 atomic absorption spectrometry. *Biol. Trace Element Res.* **118**: 146-158.
- 2187 Welch, R. M. and Norvell, W. A. 1999. Mechanisms of cadmium uptake, translocation and
2188 deposition in plants. In: McLaughlin, M. J. and Singh, B. R. (eds) Cadmium in soils
2189 and plants. Kluwer Academic Publishers, Dordrecht.
- 2190 Weng, L., Temminghoff, E. J. M., Lofts, S., Tipping, E. and Van Riemsdijk, W. H. 2002.
2191 Complexation with dissolved organic matter and solubility control of heavy metals in
2192 a sandy soil. *Environ. Sci. Technol.* **36**: 4804-4810.
- 2193 White, M. C., Chaney, R. L. and Decker, A. M. 1981. Metal complexation in xylem fluid. III.
2194 Electrophoretic evidence. *Plant Physiol.* **67**: 311-315.

- 2195 White, P. J. 2001. The pathways of calcium movement to the xylem. *J. Exp. Bot.* **358**: 892-
2196 899.
- 2197 Wiggerhauser, M., Bigalke, M., Imseng, M., Müller, M., Keller, A., Murphy, K., Kreissig,
2198 K., Rehkämper, M., Wilcke, W. and Frossard, E. 2016. Cadmium isotope fractionation
2199 in soil–wheat systems. *Environ. Sci. Technol.* **50**: 9223-9231.
- 2200 Willems, G., Dräger, D. B., Courbot, M., Godé, C., Verbruggen, N. and Saumitou-Laprade, P.
2201 2007. The genetic basis of zinc tolerance in the metallophyte *Arabidopsis halleri* ssp.
2202 *halleri* (Brassicaceae): An analysis of quantitative trait loci. *Genetics* **176**: 659-674.
- 2203 Wojas, S., Hennig, J., Plaza, S., Geisler, M., Siemianowski, O., Sklodowska, A.,
2204 Ruszczynska, A., Bulska, E. and Antosiewicz, D. M. 2009. Ectopic expression of
2205 *Arabidopsis* ABC transporter MRP7 modifies cadmium root-to-shoot transport and
2206 accumulation. *Environ. Pollut.* **157**: 2781-2789.
- 2207 Wójcik, M., Skórzyńska-Polit, E. and Tukiendorf, A. 2006. Organic acids accumulation and
2208 antioxidant enzyme activities in *Thlaspi caerulescens* under Zn and Cd stress. *Plant*
2209 *Growth Regul.* **48**: 145-155.
- 2210 Wojcik, M., Vangronsveld, J., D'Haen, J. and Tukiendorf, A. 2005a. Cadmium tolerance in
2211 *Thlaspi caerulescens*: II. Localization of cadmium in *Thlaspi caerulescens*. *Environ.*
2212 *Exp. Bot.* **53**: 163-171.
- 2213 Wojcik, M., Vangronsveld, J. and Tukiendorf, A. 2005b. Cadmium tolerance in *Thlaspi*
2214 *caerulescens*: I. Growth parameters, metal accumulation and phytochelatin synthesis
2215 in response to cadmium. *Environ. Exp. Bot.* **53**: 151-161.
- 2216 Wong, C. K. E. and Cobbett, C. S. 2009. HMA P-type ATPases are the major mechanism for
2217 root-to-shoot Cd translocation in *Arabidopsis thaliana*. *New Phytol.* **181**: 71-78.

- 2218 Wong, C. K. E., Jarvis, R. S., Sherson, S. M. and Cobbett, C. S. 2009. Functional analysis of
2219 the heavy metal binding domains of the Zn/Cd-transporting ATPase, HMA2, in
2220 *Arabidopsis thaliana*. *New Phytol.* **181**: 79-88.
- 2221 Wu, D., Yamaji, N., Yamane, M., Kashino-Fujii, M., Sato, K. and Ma, J. F. 2016a. The
2222 HvNramp5 transporter mediates uptake of cadmium and manganese, but not iron.
2223 *Plant Physiol.* **172**: 1899-1910.
- 2224 Wu, J., Sagervanshi, A. and Mühling, K. H. 2018. Sulfate facilitates cadmium accumulation
2225 in leaves of *Vicia faba* L. at flowering stage. *Ecotoxicol. Environ. Safety* **156**: 375-
2226 382.
- 2227 Wu, Q., Shigaki, T., Williams, K. A., Han, J.-S., Kim, C. K., Hirschi, K. D. and Park, S. 2011.
2228 Expression of an *Arabidopsis* Ca²⁺/H⁺ antiporter CAX1 variant in petunia enhances
2229 cadmium tolerance and accumulation. *J. Plant Physiol.* **168**: 167-173.
- 2230 Wu, Q. T. 1989. Biodisponibilité du cadmium dans les systèmes sol-plante. Ecole Nationale
2231 Supérieure d'Agronomie et des Industries Alimentaires. Institut National
2232 Polytechnique de Lorraine, Vandoeuvre-les-Nancy.
- 2233 Wu, Z., Wang, F., Liu, S., Du, Y., Li, F., Du, R., Wen, D. and Zhao, J. 2016b. Comparative
2234 responses to silicon and selenium in relation to cadmium uptake, compartmentation in
2235 roots, and xylem transport in flowering Chinese cabbage (*Brassica campestris* L. ssp.
2236 *chinensis* var. *utilis*) under cadmium stress. *Environ. Exp. Bot.* **131**: 173-180.
- 2237 Xiao, H., Yin, L., Xu, X., Li, T. and Han, Z. 2008. The iron-regulated transporter,
2238 MbNRAMP1, isolated from *Malus baccata* is involved in Fe, Mn and Cd trafficking.
2239 *Ann. Bot.* **102**: 881-889.
- 2240 Xie, H., Jiang, R., Zhang, F., McGrath, S. and Zhao, F. 2009. Effect of nitrogen form on the
2241 rhizosphere dynamics and uptake of cadmium and zinc by the hyperaccumulator
2242 *Thlaspi caerulescens*. *Plant Soil* **318**: 205-215.

- 2243 Xiong, J., An, L., Lu, H. and Zhu, C. 2009. Exogenous nitric oxide enhances cadmium
2244 tolerance of rice by increasing pectin and hemicellulose contents in root cell wall.
2245 *Planta* **230**: 755-765.
- 2246 Xu, S., Lin, S. and Lai, Z. 2015. Cadmium impairs iron homeostasis in *Arabidopsis thaliana*
2247 by increasing the polysaccharide contents and the iron-binding capacity of root cell
2248 walls. *Plant Soil* **392**: 71-85.
- 2249 Yamaguchi, C., Takimoto, Y., Ohkama-Ohtsu, N., Hokura, A., Shinano, T., Nakamura, T.,
2250 Suyama, A. and Maruyama-Nakashita, A. 2016. Effects of cadmium treatment on the
2251 uptake and translocation of sulfate in *Arabidopsis thaliana*. *Plant Cell Physiol.* **57**:
2252 2353-2366.
- 2253 Yamaji, N. and Ma, J. F. 2017. Node-controlled allocation of mineral elements in Poaceae.
2254 *Curr. Opin. Plant Biol.* **39**: 18-24.
- 2255 Yamaji, N., Xia, J., Mitani-Ueno, N., Yokosho, K. and Feng Ma, J. 2013. Preferential
2256 delivery of zinc to developing tissues in rice is mediated by P-Type Heavy Metal
2257 ATPase OsHMA2. *Plant Physiol.* **162**: 927-939.
- 2258 Yan, B.-F., Nguyen, C., Pokrovsky, O. S., Candaudap, F., Coriou, C., Bussière, S., Robert, T.
2259 and Cornu, J. Y. 2018. Contribution of remobilization to the loading of cadmium in
2260 durum wheat grains: impact of post-anthesis nitrogen supply. *Plant Soil* **424**: 591-606.
- 2261 Yan, J., Wang, P., Wang, P., Yang, M., Lian, X., Tang, Z., Huang, C. F., Salt, D. E. and Zhao,
2262 F. J. 2016. A loss-of-function allele of OsHMA3 associated with high cadmium
2263 accumulation in shoots and grain of Japonica rice cultivars. *Plant Cell Environ.* **39**:
2264 1941-1954.
- 2265 Yang, M., Zhang, Y., Zhang, L., Hu, J., Zhang, X., Lu, K., Dong, H., Wang, D., Zhao, F.-J.,
2266 Huang, C.-F. and Lian, X. 2014. OsNRAMP5 contributes to manganese translocation
2267 and distribution in rice shoots. *J. Exp. Bot.* **65**: 4849-4861.

- 2268 Yang, X. E., Long, X. X., Ye, H. B., He, Z. L., Calvert, D. V. and Stoffella, P. J. 2004.
 2269 Cadmium tolerance and hyperaccumulation in a new Zn-hyperaccumulating plant
 2270 species (*Sedum alfredii* Hance). *Plant Soil* **259**: 181-189.
- 2271 Yang, Y., Xiong, J., Chen, R., Fu, G., Chen, T. and Tao, L. 2016. Excessive nitrate enhances
 2272 cadmium (Cd) uptake by up-regulating the expression of OsIRT1 in rice (*Oryza*
 2273 *sativa*). *Environ. Exp. Bot.* **122**: 141-149.
- 2274 Yoneyama, T., Gosho, T., Kato, M., Goto, S. and Hayashi, H. 2010. Xylem and phloem
 2275 transport of Cd, Zn and Fe into the grains of rice plants (*Oryza sativa* L.) grown in
 2276 continuously flooded Cd-contaminated soil. *Soil Sci. Plant Nutr.* **56**: 445-453.
- 2277 Yu, H.-Y., Liu, C., Zhu, J., Li, F., Deng, D.-M., Wang, Q. and Liu, C. 2016. Cadmium
 2278 availability in rice paddy fields from a mining area: The effects of soil properties
 2279 highlighting iron fractions and pH value. *Environ. Pollut.* **209**: 38-45.
- 2280 Zhang, F., Römheld, V. and Marschner, H. 1989. Effect of zinc deficiency in wheat on the
 2281 release of zinc and iron mobilizing root exudates. *Z. Pflanzenernaehrung Bodenkunde*
 2282 **152**: 205-210.
- 2283 Zhang, L., Wu, J., Tang, Z., Huang, X.-Y., Wang, X., Salt, D. E. and Zhao, F.-J. 2019.
 2284 Variation in the BrHMA3 coding region controls natural variation in cadmium
 2285 accumulation in *Brassica rapa* vegetables. *J. Exp. Bot.* **70**: 5865-5878.
- 2286 Zhang, X. D., Wang, Y., Li, H. B. and Yang, Z. M. 2018. Isolation and identification of
 2287 rapeseed (*Brassica napus*) cultivars for potential higher and lower Cd accumulation. *J.*
 2288 *Plant Nutr. Soil Sci.* **181**: 479-487.
- 2289 Zhang, Z.-C., Chen, B.-X. and Qiu, B.-S. 2010. Phytochelatin synthesis plays a similar role in
 2290 shoots of the cadmium hyperaccumulator *Sedum alfredii* as in non-resistant plants.
 2291 *Plant Cell Environ.* **33**: 1248-1255.

- 2292 Zhao, F. J., Hamon, R. E. and McLaughlin, M. J. 2001. Root exudates of the
 2293 hyperaccumulator *Thlaspi caerulescens* do not enhance metal mobilization. *New*
 2294 *Phytol.* **151**: 613-620.
- 2295 Zhou, H., Zeng, M., Zhou, X., Liao, B.-H., Peng, P.-Q., Hu, M., Zhu, W., Wu, Y.-J. and Zou,
 2296 Z.-J. 2015. Heavy metal translocation and accumulation in iron plaques and plant
 2297 tissues for 32 hybrid rice (*Oryza sativa* L.) cultivars. *Plant Soil* **386**: 317-329.
- 2298 Zhou, H., Zhu, W., Yang, W.-T., Gu, J.-F., Gao, Z.-X., Chen, L.-W., Du, W.-Q., Zhang, P.,
 2299 Peng, P.-Q. and Liao, B.-H. 2018. Cadmium uptake, accumulation, and remobilization
 2300 in iron plaque and rice tissues at different growth stages. *Ecotoxicol. Environ. Safety*
 2301 **152**: 91-97.
- 2302 Zimeri, A. M., Dhankher, O. P., McCaig, B. and Meagher, R. B. 2005. The plant MT1
 2303 metallothioneins are stabilized by binding cadmiums and are required for cadmium
 2304 tolerance and accumulation. *Plant Mol. Biol.* **58**: 839-855.
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2309 **Figure captions**

2310 **Figure 1.** Principal reactions and processes controlling cadmium availability at the root-soil
2311 interface. PM: Plasma membrane; L_s: Organic ligand originating from the soil; L_p: organic
2312 ligand originating from rhizodeposition. For simplification, the cell wall is not represented.

2313 **Figure 2.** Schematic representation of *Arabidopsis thaliana* root with the gene/proteins
2314 involved in Cd transport. For simplification, the cell wall is not represented.

2315 **Figure 3.** Schematic representation of a rice (*Oryza sativa*) root with the gene/proteins
2316 involved in Cd transport. For simplification, the cell wall is not represented. Question mark
2317 after a protein name means it might not be expressed in the tissue, although it has been found
2318 in rice roots.

2319 **Figure 4.** Schematic representation of *Noccaea caerulescens* root with the gene/proteins
2320 involved in Cd transport. For simplification, the cell wall is not represented.

2321 **Figure 5.** Schematic representation of leaf tissues with the transporters involved in Cd
2322 distribution. The prefix before the gene name indicates the plant species in which it was
2323 observed. Nc: *Noccaea caerulescens*, Os: *Oryza sativa*. No prefix means that the
2324 gene/transport protein has been observed in several plant species. For simplification, the cell
2325 wall is not represented.

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2331 **Table 1.** Genes involved in Cd accumulation in plants.

Function	Name	Annotation	Organ of expression	Plant species	Co-transported	Factor of upregulation	References
Uptake into cell	IRT1	ZIP family	Roots	<i>Pisum sativum</i> , <i>Arabidopsis thaliana</i> , <i>Hordeum vulgare</i> , <i>Oryza sativa</i>	Co ²⁺ , Fe ²⁺ , Fe ³⁺ , Mn ²⁺ , Zn ²⁺	Fe deficiency	Cohen <i>et al.</i> (1998); Korshunova <i>et al.</i> (1999); Nakanishi <i>et al.</i> (2006); Pedas <i>et al.</i> (2008); Barberon <i>et al.</i> (2014)
	IRT2		Roots	<i>O. sativa</i>	Fe ²⁺	Fe deficiency	Nakanishi <i>et al.</i> (2006)
	ZNT1		Roots, shoot	<i>Noccaea caerulea</i>	Zn ²⁺	Zn deficiency	Pence <i>et al.</i> (2000); Milner <i>et al.</i> (2012); Lin <i>et al.</i> (2016a)
	NRAMP1	natural resistance-associated macrophage protein	Roots(+), shoot(-)	<i>A. thaliana</i> ¹ , <i>O. sativa</i> , <i>Malus baccata</i>	Fe, Mn	Fe and Mn deficiency	Thomine <i>et al.</i> (2000); Xiao <i>et al.</i> (2008); Cailliatte <i>et al.</i> (2010); Takahashi <i>et al.</i> (2011); Ishimaru <i>et al.</i> (2012)
	NRAMP5		Roots	<i>O. sativa</i> , <i>Triticum polonicum</i> , <i>T. aestivum</i> , <i>Zea mays</i>	Mn, Fe, Co		Ishimaru <i>et al.</i> (2012); Sasaki <i>et al.</i> (2012); Tang <i>et al.</i> (2017); Sui <i>et al.</i> (2018)
	GLR3.4	glutamate receptors	Roots	<i>O. sativa</i>	Ca		Chen <i>et al.</i> (2018b)
	ANN4	annexin	Roots	<i>O. sativa</i>	Ca		Chen <i>et al.</i> (2018b)
Efflux out of cell	MTP9	cation diffusion facilitator (CDF)	Root endodermis	<i>Cucumis sativus</i>	Mn	Mn and Cd excess	Migocka <i>et al.</i> (2015)
	PDR8	ATP binding cassettes (ABC)	Roots(++) (hair and	<i>A. thaliana</i>	Pb ²⁺		Kim <i>et al.</i> (2007)

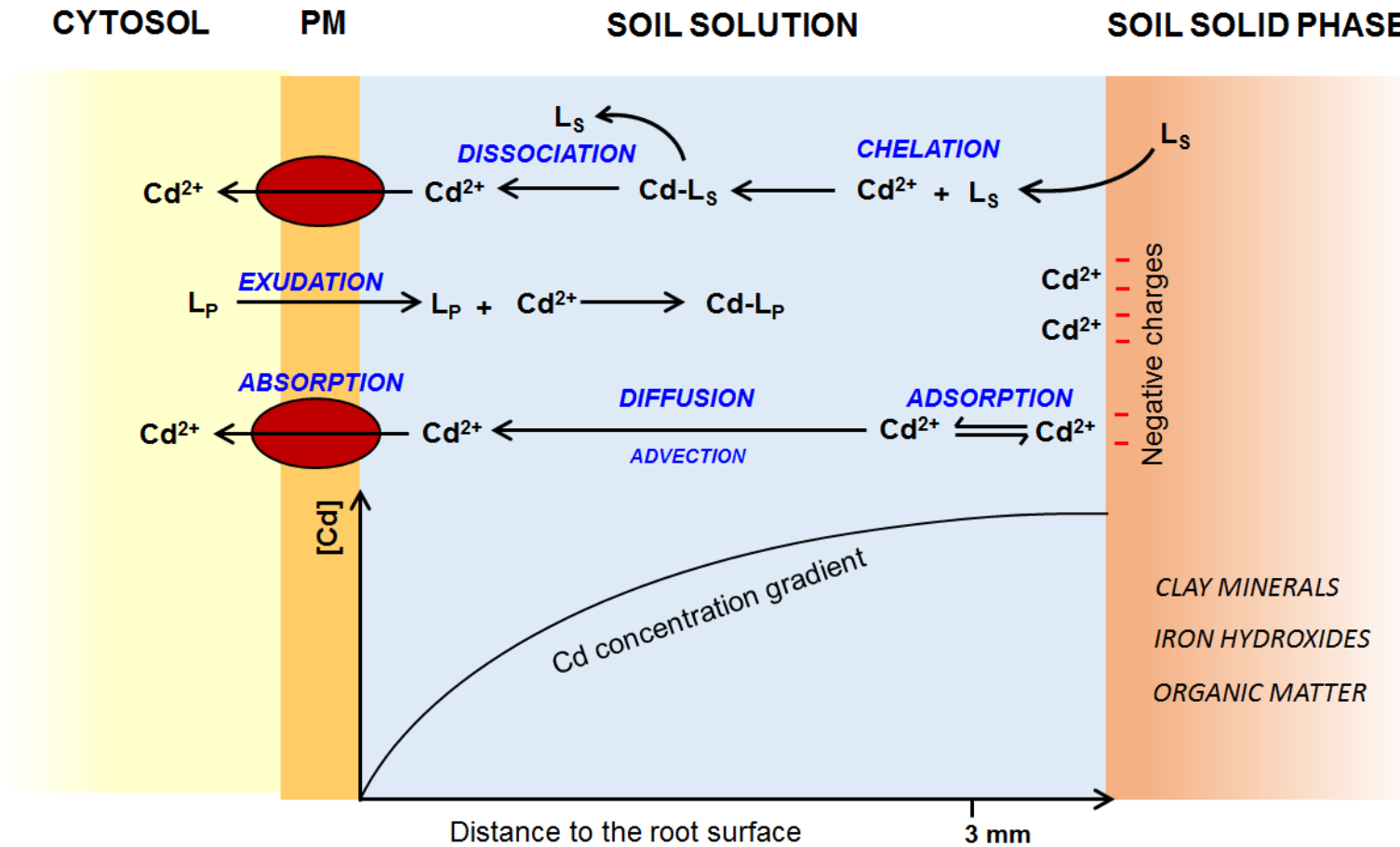
			epidermis), shoot				
Chelation	MTL	MT-like protein	Roots	<i>Sedum plumbizincicola</i>			Peng <i>et al.</i> (2017a)
	CDT1	Cys-rich peptide	Roots, shoots (cell surface, cell wall)	<i>Digitaria ciliaris</i> , <i>O. sativa</i>		Cd exposure	Kuramata <i>et al.</i> (2009)
	CAL1	Defensin like protein	Roots	<i>O. sativa</i>			Luo <i>et al.</i> (2018)
Uptake into the vacuole	HMA3	P type ATPase family, subfamily HMA (for Heavy Metal Associated)	Roots, shoot	<i>A. thaliana</i> , <i>O. sativa</i> , <i>N. caerulea</i> , <i>S. plumbizincicola</i> , <i>Brassica rapa</i> , <i>Triticum durum</i>	Co, Pb, Zn		Morel <i>et al.</i> (2009); Miyadate <i>et al.</i> (2011); Ueno <i>et al.</i> (2011a); Ueno <i>et al.</i> (2011b); Yan <i>et al.</i> (2016); Liu <i>et al.</i> (2017); Shao <i>et al.</i> (2018); Maccaferri <i>et al.</i> (2019); Sui <i>et al.</i> (2019); Zhang <i>et al.</i> (2019)
	MRP3	ATP binding cassettes (ABC)	Roots, shoot	<i>A. thaliana</i> ¹ , <i>H. vulgare</i>			Tommasini <i>et al.</i> (1998); Schneider <i>et al.</i> (2009)
	MRP7		Roots, shoot	<i>A. thaliana</i> ²			Wojas <i>et al.</i> (2009)
	ABCC1		Roots, shoot	<i>A. thaliana</i> , <i>H. vulgare</i>	As		Park <i>et al.</i> (2012); Song <i>et al.</i> (2014)
	ABCC2		Roots, shoot	<i>A. thaliana</i> , <i>H. vulgare</i>	As		Park <i>et al.</i> (2012); Song <i>et al.</i> (2014)
	ABCC3		Roots, shoot	<i>A. thaliana</i>		Cd exposure	Brunetti <i>et al.</i> (2015)
	CAX2	CAtion eXchanger	Roots, shoot	<i>A. thaliana</i> ²	Ca ²⁺ , Zn ²⁺ , Mn ²⁺		Pittman <i>et al.</i> (2004); Koren'kov <i>et al.</i> (2007)
	CAX4		Roots, shoot	<i>A. thaliana</i> ²	Ca ²⁺ , Zn ²⁺ , Mn ²⁺		Koren'kov <i>et al.</i> (2007)

Efflux out of the vacuole	NRAMP3	natural resistance-associated macrophage protein	Roots, shoot	<i>A. thaliana</i> , <i>N. caerulescens</i>	Fe, Mn	Fe deficiency	Oomen <i>et al.</i> (2009) Thomine <i>et al.</i> (2000)
	NRAMP4		Roots, shoot	<i>A. thaliana</i> , <i>N. caerulescens</i>	Fe, Mn, Zn	Fe deficiency	Oomen <i>et al.</i> (2009) Thomine <i>et al.</i> (2000)
Xylem loading	HMA2	P type ATPase family, subfamily HMA (for Heavy Metal Associated)	Roots(++), shoot	<i>A. thaliana</i> , <i>O. sativa</i>	Zn		Wong and Cobbett (2009); Wong <i>et al.</i> (2009); Nocito <i>et al.</i> (2011); Satoh-Nagasawa <i>et al.</i> (2012); Takahashi <i>et al.</i> (2012); Yamaji <i>et al.</i> (2013)
	HMA4		Roots(++), shoot	<i>A. thaliana</i> , <i>A. halleri</i> , <i>N. caerulescens</i>	Zn, Pb, Cu	Zn and Mn exposure, downregulation by Cd exposure	Papoyan and Kochian (2004); Verret <i>et al.</i> (2004); Mills <i>et al.</i> (2005); Courbot <i>et al.</i> (2007); Hanikenne <i>et al.</i> (2008); Ó Lochlainn <i>et al.</i> (2011); Craciun <i>et al.</i> (2012)
	CCX2	cation/calcium (Ca) exchanger	Root, Shoot (node++)	<i>O. sativa</i>	Ca		Hao <i>et al.</i> (2018)
Phloem loading	LCT1	low affinity cation transporter	Shoot	<i>O. sativa</i>	K, Mg, Ca, Mn		Uraguchi <i>et al.</i> (2011); Uraguchi <i>et al.</i> (2014)
	HMA2	P type ATPase family, subfamily HMA (for Heavy Metal Associated)	Shoot (node)	<i>O. sativa</i>			Yamaji and Ma (2017)

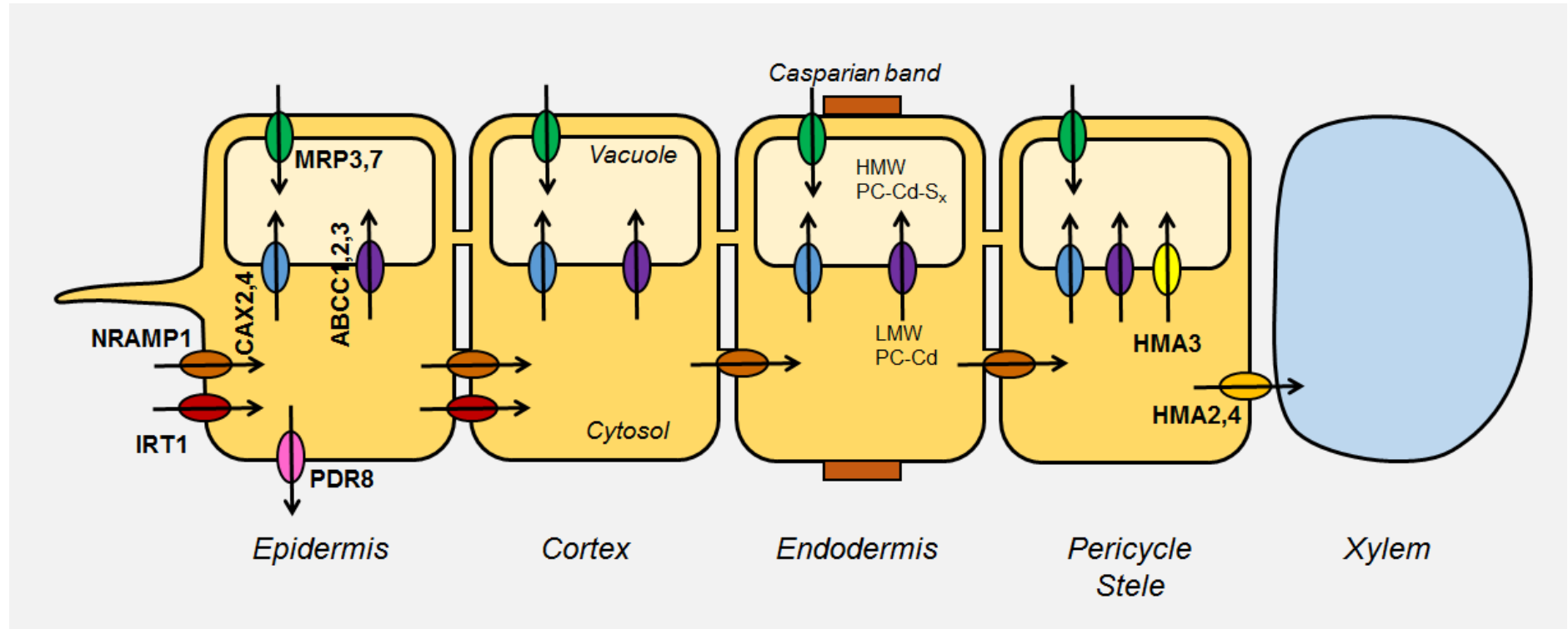
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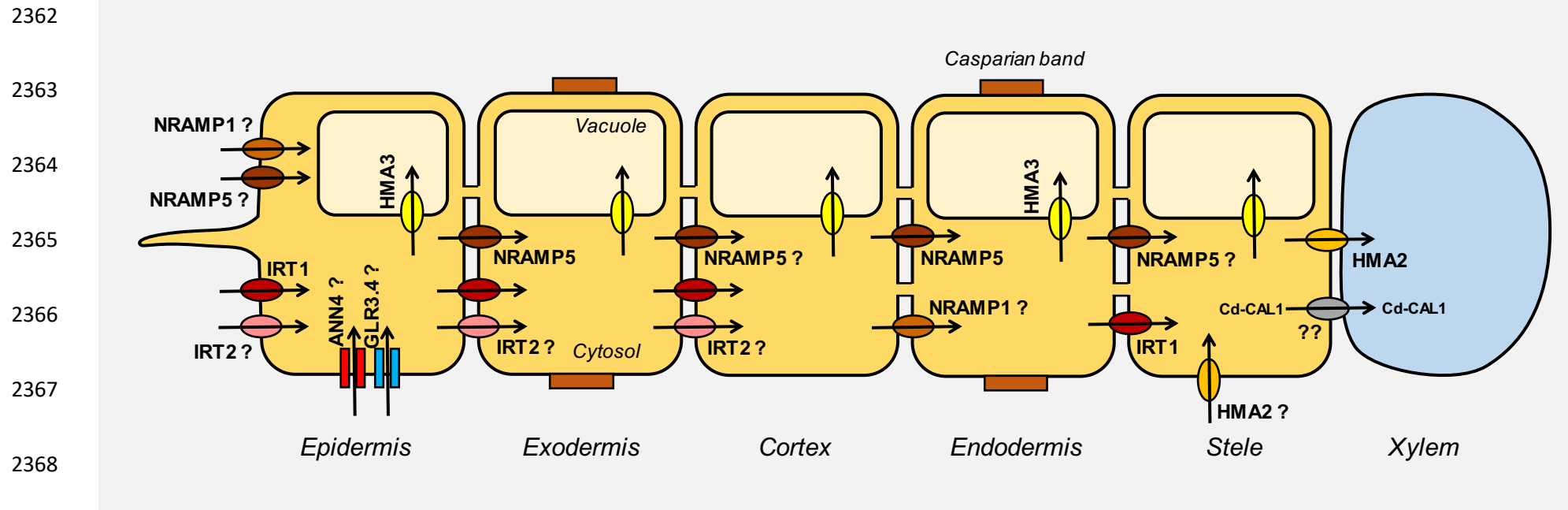
Figure 1. Principal reactions and processes controlling cadmium availability at the root-soil interface. PM: Plasma membrane; L_s: Organic ligand originating from the soil; L_p: organic ligand originating from rhizodeposition. For simplification, the cell wall is not represented.



2348 **Figure 2.** Schematic representation of *Arabidopsis thaliana* root with the gene/proteins involved in Cd transport. For simplification, the cell wall
 2349 is not represented.



2360 **Figure 3.** Schematic representation of a rice (*Oryza sativa*) root with the gene/proteins involved in Cd transport. For simplification, the cell wall
 2361 is not represented. A question mark after a protein name means it might not be expressed in the tissue, although it has been found in rice roots.



2372 **Figure 4.** Schematic representation of the hyperaccumulator *Noccaea caerulescens* root with the gene/proteins involved in Cd transport. For
2373 simplification, the cell wall is not represented.

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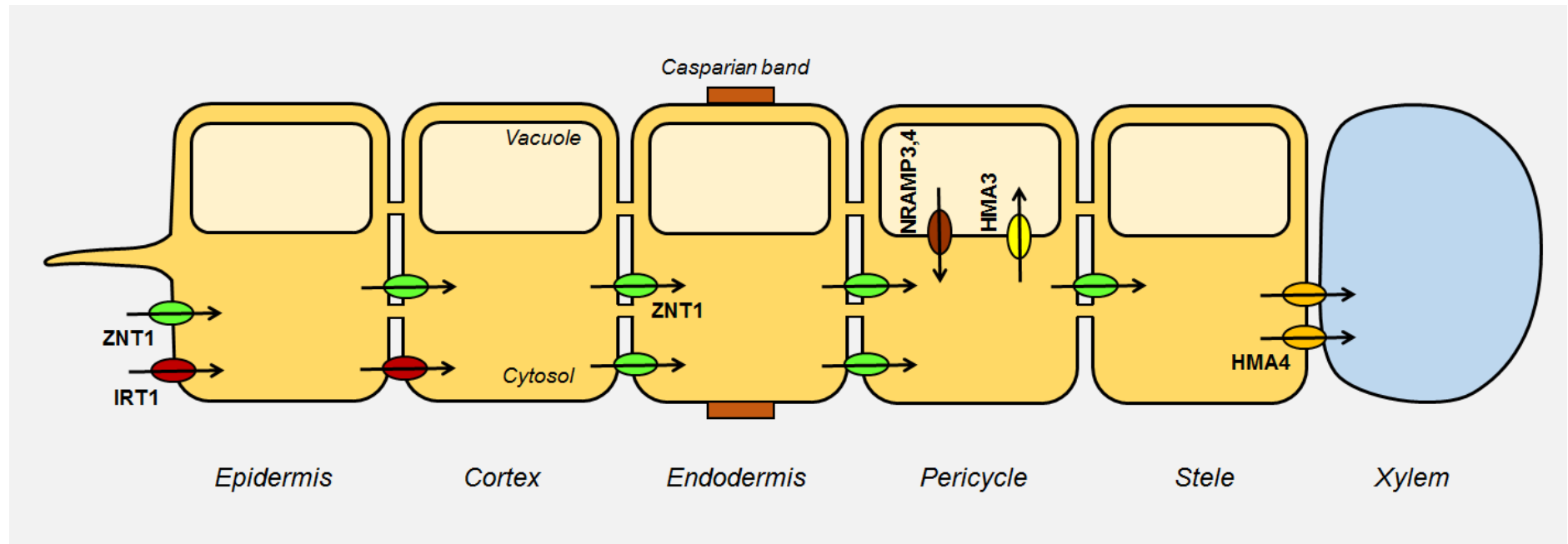
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2383 **Figure 5.** Schematic representation of leaf tissues with the transporters involved in Cd distribution. The prefix before the gene name indicates the
 2384 plant species in which it was observed. Nc: *Noccaea caerulescens*, Os: *Oryza sativa*. No prefix means that the gene/transport protein has been
 2385 observed in several plant species. For simplification, the cell wall is not represented.

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