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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

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

54 Apart from tobacco smoking, food is the main source of human exposure to Cd, through  
55 cereals, vegetables, roots and tubers (EFSA, 2012). In these plant products, Cd comes from  
56 the soil, through root absorption and translocation to the harvested organs. Most of the soil Cd  
57 originates from contamination resulting from human activities. The sources of contamination  
58 are atmospheric deposition of industrial and urban emissions, phosphate fertilizers, organic  
59 and mineral amendments. In Europe, as atmospheric deposition has decreased since the mid-  
60 1960s, Cd from P fertilizer is now the major input into cultivated soils (Six and Smolders,  
61 2014; Sterckeman *et al.*, 2018). The dominant form of the metal in soil is  $\text{Cd}^{2+}$ . The physico-  
62 chemical properties of this ion lead to its accumulation in the soil's ploughed layer by binding  
63 to soil solid components. However, this binding is largely reversible (Smolders *et al.*, 1999),  
64 making Cd one of most available trace metals for root absorption after desorption into the soil  
65 solution (Lin *et al.*, 2016b).

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

73 Cadmium chemistry in soil and its accumulation in plants have been studied since the  
74 seventies (*e.g.* (Lagerwerff, 1971; John *et al.*, 1972; Haghiri, 1973). The aim of the  
75 investigations carried out until now has been to understand the soil supply of Cd to roots as  
76 well as the distribution, the toxicity and the tolerance of the metal in the plant organs. Several  
77 works have shown that Cd distribution in the plant varies greatly both from one species to  
78 another and within a given species, depending on the population or the cultivar (Florijn and  
79 Van Beusichem, 1993b; Greger and Lofstedt, 2004; Gonneau *et al.*, 2014; Laporte *et al.*,  
80 2015). This highlights the importance of genetic factors in this distribution. Over the two last  
81 decades, numerous publications have addressed the biomolecular processes of Cd  
82 accumulation in plants, with a particular effort being made to identify the genes and related  
83 proteins responsible for Cd uptake, transport, storage and detoxification in plant tissues.

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

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

96 We have written this review bearing in mind these underlying applications, which both  
97 require an in-depth understanding of the mechanisms controlling Cd flow from soil to plant,  
98 in order to be able to either reduce the Cd content in the consumed parts (grain, tubers, leaves)  
99 or increase it in the harvested shoots of the plant used to decontaminate the soils. This review  
100 thus focuses both on the processes responsible for the transfer of Cd from soil to plant and on  
101 those responsible for the distribution of the metal in the different plant parts under non-toxic  
102 conditions. The mechanisms of Cd toxicity and tolerance are not addressed as such in this  
103 review, but only with regards to their possible contribution to the flow and the distribution of  
104 the metal in the plant. For instance, Cd sequestration in cell walls and in vacuoles is a way to  
105 reduce Cd toxicity in plants, but it will be regarded here more as a factor controlling Cd  
106 distribution and storage among tissues and organs. The reader may refer to other literature  
107 reviews regarding the management of Cd toxicity by plants (Moullis, 2010; Lin and Aarts,  
108 2012; Verbruggen *et al.*, 2013; Choppala *et al.*, 2014; Kupper and Andresen, 2016).

## 109 **I. Overview of the plant Cd accumulation processes**

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

115 surface, generating a concentration gradient, which in turn drives diffusion of  $\text{Cd}^{2+}$  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,  $\text{Cd}^{2+}$   
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  $\text{Cd}^{2+}$  may accumulate passively by 4 orders of magnitude. Therefore, active transport is not  
130 required to drive  $\text{Cd}^{2+}$  uptake into root cells. Once in the cytosol, Cd ions are complexed by  
131 organic ligands. This reduces the concentration of free  $\text{Cd}^{2+}$  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  $\text{Cd}^{2+}$  is absorbed into the root symplast, its  
139 translocation to the aerial parts is governed by three main processes: sequestration in the root

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

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

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

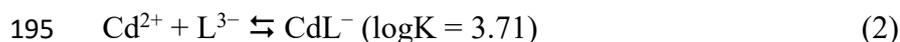
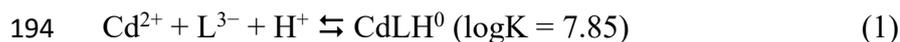
## 161 II. Chemistry of Cd in the rhizosphere

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

164 concentrations of the metal in solution. These reactions take place in the soil solution and at  
165 the surface of the solid phase (Figure 1). With an ionic potential ( $IP = Z/r$ , the valence divided  
166 by the ionic radius in nm) of  $21 \text{ nm}^{-1}$ ,  $\text{Cd}^{2+}$  is a solvating cation, whose behaviour can be  
167 differentiated from that of hydrolysing cations, such as  $\text{Al}^{3+}$  ( $IP = 56 \text{ nm}^{-1}$ ), and oxyanion-  
168 forming cations, such as  $\text{Mo}^{6+}$  ( $IP = 143 \text{ nm}^{-1}$ ) (Sposito, 1989). Based on this parameter,  $\text{Cd}^{2+}$   
169 behaves similarly to  $\text{Ca}^{2+}$  ( $IP = 20 \text{ nm}^{-1}$ ). However, it is differentiated from this cation by its  
170 Misono softness parameter ( $Y$ , nm), whose value is 0.31 nm (Misono *et al.*, 1967; Sposito,  
171 1989). Values of  $Y$  above 0.32 nm correspond to metal ions with high polarizability and low  
172 electronegativity, which tend to form covalent chemical bonds, while values below 0.25 nm  
173 correspond to ions with low polarizability and high electronegativity tending to form ionic  
174 bonds. Cadmium, together with  $\text{Cu}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Ni}^{2+}$  and  $\text{Fe}^{2+}$  fall into the “borderline” zone  
175 ( $0.25 < Y < 0.32$ ), while  $\text{Ca}^{2+}$  has a  $Y$  value of 0.16 nm and Hg and Pb have  $Y$  values of 0.40  
176 nm. This makes  $\text{Cd}^{2+}$  chemical behaviour intermediate between that of  $\text{Ca}^{2+}$ , forming ionic  
177 bonds, and those of  $\text{Pb}^{2+}$  and  $\text{Hg}^{2+}$ , often involved in covalent bonds.

178 In the solution of non- or low contaminated soils, Cd concentration is generally below 10 nM,  
179 while in Cd polluted soils, it ranges between 10 nM to 200 nM, and can even reach  
180 micromolar concentrations under extreme contamination and solubility conditions (Helmke,  
181 1999; Sauvé *et al.*, 2000b; Weng *et al.*, 2002; Nolan *et al.*, 2003; Maxted *et al.*, 2007). The  
182 free hydrated ion ( $\text{Cd}^{2+}$ ) is in equilibrium with organic and inorganic complexes.  $\text{Cd}^{2+}$  reacts  
183 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  
184 chloride, particularly in salt affected soils,  $\text{Cd}^{2+}$  can form soluble chloride complexes, i.e.  
185  $\text{CdCl}^+$ ,  $\text{CdCl}_2$ ,  $\text{CdCl}_3^-$  and  $\text{CdCl}_4^{2-}$ .  $\text{Cd}^{2+}$  reacts with sulphate, to form  $\text{CdSO}_4$  complexes and,  
186 in alkaline soil, with carbon dioxide to form  $\text{CdCO}_3$  and  $\text{CdHCO}_3^+$  in solution (Alloway,  
187 1995; Helmke, 1999). Free  $\text{Cd}^{2+}$  is considered to dominate in soil solutions (Degryse *et al.*,  
188 2009; Ren *et al.*, 2015a), although some measurements and modelling have shown that this is

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



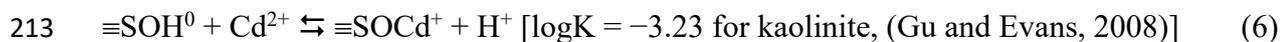
198 Cadmium competes with other cations, such as  $\text{Ca}^{2+}$  for complexation with soluble organic  
 199 ligands (Lin *et al.*, 2016b).

200 In most soils, the amount of dissolved Cd is much lower than that bound to the solid phase.  
 201 Cadmium can precipitate as carbonate or phosphate, but soils are generally under-saturated  
 202 with these minerals (Tye *et al.*, 2003). Even if super-saturated, dissolved organic matter has  
 203 been reported to inhibit  $\text{CdCO}_3$  precipitation (Holm *et al.*, 1996). Therefore, rather than  
 204 precipitation, adsorption on mineral surfaces and binding to humic substances are the  
 205 processes which control Cd partitioning between solid and liquid phases (Lofts and Tipping,  
 206 1998; Tipping, 1998; Ren *et al.*, 2015b). Indeed, Cd can bind with particulate fulvic or humic  
 207 acid (R) with a net charge Z, exchanging against protons according to the following equation:



209 The metal also interacts with the surface sites on minerals, particularly on Fe and Mn  
 210 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,  $K_d$  ( $\text{L. kg}^{-1}$ ), 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

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

224 proposed by Degryse *et al.* (2009),  $C_{\text{org}}$  being the organic carbon content (%) and  $pH_{\text{CaCl}_2}$  the  
 225 pH of a soil suspension in a 0.01 M  $\text{CaCl}_2$  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  $\text{Cd}^{2+}$  replacing  $\text{Ca}^{2+}$ . 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  $\text{Cd}^{2+}$ . Eqs. 5-8 indicate that protons

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

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

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

### 249 **III. Cadmium species absorbed by the roots**

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

257 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<sup>2+</sup>.

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; Schaidler *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<sup>2+</sup>, 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*  
280 *al.*, 2004). Two mechanisms could account for this effect: (i) the formation of CdCl<sub>n</sub><sup>2-n</sup>  
281 complexes could favour the desorption of the metal from the soil solid phase, (ii) the  
282 complexes (CdCl<sub>n</sub><sup>2-n</sup>) could diffuse faster than the free ion in the rhizosphere or in the

283 apoplast. Absorption of the complexes themselves is not excluded, as  $\text{CdCl}^+$  could cross the  
284 plasma membrane through selective ion channels for monovalent cations such as  $\text{K}^+$ . In  
285 addition, the neutral form  $\text{CdCl}_2^0$  could passively cross the lipid bilayer (Welch and Norvell,  
286 1999).

287 Analogous effects, but less intense than with  $\text{Cl}^-$ , have been obtained with  $\text{SO}_4^{2-}$ . The Cd  
288 concentration in the soil solution increases upon addition of sulphate, which slightly increases  
289 Cd absorption (McLaughlin *et al.*, 1998b). An experiment in hydroponics suggests that the  
290  $\text{CdSO}_4^0$  complex is taken up by plants as efficiently as free  $\text{Cd}^{2+}$  ion (McLaughlin *et al.*,  
291 1998a). The electrically neutral  $\text{CdSO}_4^0$  complex would passively diffuse across the plasma  
292 membrane.

#### 293 **IV. Influence of the plant on soil Cd availability**

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

301 However, this effect could only rarely be demonstrated in the case of Cd. Wu (1989) found  
302 that nitrogen fertilization of ryegrass using  $\text{NH}_4^+$  increased plant Cd uptake, compared with  
303 fertilization using nitrate. He attributed this observation to the increase in soil acidity  
304 following the higher proton release by the roots absorbing ammonium.

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

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

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

330 Phytosiderophores, such as mugineic and deoxy mugineic acids (MA, DMA), are iron-  
331 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.

## 356 V. Absorption of Cd by the roots

### 357 A. Location of Cd uptake on the root

358 Laporte *et al.* (2013) showed that in sunflower first order lateral roots, Cd<sup>2+</sup> influx was 2.9  
359 times higher at root tip than at the root base. These results are consistent with those of Pineros  
360 *et al.* (1998), who observed significantly higher Cd influx at positions 1 to 1.5 mm from the  
361 root apex of wheat and *N. caerulescens* seedlings. Similar profiles were observed in various  
362 young roots, in durum wheat (*T. turgidum* L. ssp. *durum*) (Farrell *et al.*, 2005), in poplar  
363 (*Populus* × *canescens* plantlets) (He *et al.*, 2011), in rice (Chen *et al.*, 2018b) and in the  
364 halophyte *Suaeda salsa* (Li *et al.*, 2012a). These profiles can be attributed to a higher activity  
365 of the transport systems close to the root tip, where they could be expressed at higher levels  
366 (Chen *et al.*, 2018b) and/or benefit from more energy (Laporte *et al.*, 2013). Higher Cd influx  
367 at the root apex could also result from the incomplete development of apoplastic barriers,  
368 thereby favouring apoplastic Cd uptake (Redjala *et al.*, 2011) in this part of the root. The  
369 Casparian strips and suberin lamellae in the endodermis and exodermis are formed away from  
370 the root apex in more differentiated parts of the roots. However, according to the modelling  
371 carried out by Laporte *et al.* (2013), the higher influx at the root tip would not significantly  
372 influence the overall Cd root uptake, whereas the total root surface area is indeed a major  
373 parameter.

### 374 B. Mechanisms of Cd root absorption

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

381 for essential elements with insufficient selectivity with respect to their substrate (Clemens,  
382 2001).

383 Cadmium transport into roots was first studied through absorption kinetics, formalized using  
384 the Michaelis-Menten model (Cataldo *et al.*, 1983; Costa and Morel, 1994; Cohen *et al.*,  
385 1998; Hart *et al.*, 1998; Redjala *et al.*, 2010a). Although considered as not Cd specific, the  
386 transport systems involved show a high affinity to the metal, with  $K_m$  falling in the range of  
387 0.02 to 1  $\mu\text{M}$ . For instance, the  $K_m$  values for Cd were 0.059  $\mu\text{M}$  and 0.067  $\mu\text{M}$  in bread  
388 wheat and durum wheat, respectively, whereas for Zn, a micro-nutrient, they were 2.3  $\mu\text{M}$  and  
389 3.3  $\mu\text{M}$  (Hart *et al.*, 2002). The maximum influx ( $V_{max}$ ) values ranged from 12 to 188 nmol  
390 (gFW)<sup>-1</sup> h<sup>-1</sup> (Lux *et al.*, 2011). The highest values were found for the Ganges populations of  
391 the metal hyperaccumulator *N. caerulescens* under Fe deficient conditions (Lombi *et al.*,  
392 2002). Cohen *et al.* (1998) also found that Fe deficiency stimulated high affinity Cd uptake in  
393 *Pisum sativum* roots. Lombi *et al.* (2001) measured the  $K_m$  and  $V_{max}$  of the Prayon (low Cd  
394 accumulator) and Ganges (high Cd accumulators) populations of *N. caerulescens*. They found  
395 identical  $K_m$  values (around 0.2  $\mu\text{M}$ ), but the  $V_{max}$  of the Ganges population (160 nmol (gFW)<sup>-1</sup>  
396 h<sup>-1</sup>) was significantly higher than that of the Prayon population (33 nmol (gFW)<sup>-1</sup> h<sup>-1</sup>).  
397 Similar  $V_{max}$  differences were found in the comparison between Calamine (Viviez) and  
398 Serpentine (Vosges) populations of the same species (Redjala *et al.*, 2009). Since the  $V_{max}$   
399 value is proportional to the density of membrane transporters, the difference in Cd uptake  
400 ability by the Prayon and Ganges populations could be due to a difference in the expression of  
401 a certain transporter gene with high Cd affinity.

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

406 interpretation was invalidated using Cd measurement in the symplasm and apoplasm [Redjala  
407 (2009); Redjala *et al.* (2010a)]. The linear component would rather correspond to the entry of  
408 Cd into the symplasm *via* low affinity transport systems, such as ion channels for  $\text{Ca}^{2+}$   
409 (Perfus-Barbeoch *et al.*, 2002; Rodriguez-Hernandez *et al.*, 2015; Chen *et al.*, 2018b). In any  
410 case, this linear component significantly contributes to the root uptake only at high  
411 concentrations of  $\text{Cd}^{2+}$  in the medium, which do not occur in agricultural soil and are rare in  
412 contaminated soils.

413 In mouse-ear cress (*Arabidopsis thaliana*) (Figure 2, Table 1), Eide *et al.* (1996) showed that  
414 Fe deficiency induces the expression of an iron regulated transporter IRT1 (from the zinc-  
415 regulated transporter, iron-regulated transporter protein - ZIP family), which allows the  
416 transport of divalent metals such as  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Mn}^{2+}$  and  $\text{Zn}^{2+}$ , in addition to  $\text{Fe}^{2+}$ , when  
417 expressed in yeast (Eide *et al.*, 1996; Korshunova *et al.*, 1999). AtIRT1 is mainly expressed in  
418 root epidermis and to a lesser extent in root cortex (Vert *et al.*, 2002). Cd accumulation is  
419 strongly reduced in an *irt1* knockout mutant under Fe deficient conditions (Vert *et al.*, 2002).  
420 Cohen *et al.* (1998) proposed that the up-regulation of an IRT1 orthologue accounts for  
421 elevated Cd uptake under Fe deficiency in *Pisum sativum*. Even though the IRT1 homologue  
422 in barley is also able to transport  $\text{Fe}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Cd}^{2+}$  when expressed in yeast (Pedas *et al.*  
423 (2008); (Wu *et al.*, 2016a), *in planta*, HvIRT1 mainly controls  $\text{Mn}^{2+}$  uptake (Long *et al.*,  
424 2018). In rice (Figure 3, Table 1), OsIRT1, as well as its homologue OsIRT2, are  
425 predominantly expressed in roots in Fe deficient conditions and are able to transport  $\text{Cd}^{2+}$   
426 when expressed in yeast. Iron deficiency both enhanced their expression as well as Cd uptake  
427 and translocation (Ishimaru *et al.*, 2006; Nakanishi *et al.*, 2006). IRT1 may also be involved  
428 in Cd uptake by the hyperaccumulator *N. caerulescens* (Lombi *et al.*, 2002; Plaza *et al.*,  
429 2007), but whether its activity accounts for the particularly high ability of the Ganges  
430 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

456 including grain. In *Triticum polonicum*, TpNRAMP5 is mainly expressed in roots and basal  
457 stems, and the protein is localized in the plasma membrane. Expression of TpNRAMP5 in *A.*  
458 *thaliana* enhanced the accumulation of Cd, Co and Mn, but not that of Zn and Fe (Peng *et al.*,  
459 2018). The NRAMP5 transporter is also present in maize and in bread wheat, but displays a  
460 lower level of expression and a lower activity than in rice (Sui *et al.*, 2018).

461 Several root metal uptake transporters display a polar localization. OsNRAMP5 and AtIRT1,  
462 that have been shown to mediate Cd uptake are concentrated on the membrane facing the soil  
463 solution (Sasaki *et al.*, 2012; Barberon *et al.*, 2014). This raises the question of how metals are  
464 transferred towards the stele, from the other side of the cell facing the central cylinder. In  
465 plants, members of the cation diffusion facilitator (CDF) family are known as metal tolerance  
466 proteins (MTP). They are involved in metal efflux from the cytosol to other compartments:  
467 the apoplast, the vacuole and the Trans Golgi Network (TGN). In rice, Ueno *et al.* (2015)  
468 showed that OsMTP9 is polarized on the side of the plasma membrane facing the stele and is  
469 required for efficient Mn uptake (Table 1). Working on cucumber (*Cucumis sativus*),  
470 Migocka *et al.* (2015) found that the CsMTP9 gene encodes a plasma membrane H<sup>+</sup>-coupled  
471 Mn<sup>2+</sup> and a Cd<sup>2+</sup> antiporter expressed exclusively in roots. CsMTP9 was shown to be involved  
472 in the efflux of Mn and Cd from root cells, thereby allowing the transport of both metals from  
473 the endodermis into the vascular cylinder for further translocation to the shoot.

474 Some authors have suggested that Cd could also enter cells through Ca<sup>2+</sup> channels (Welch and  
475 Norvell, 1999; Perfus-Barbeoch *et al.*, 2002; Li *et al.*, 2012b). This is supported both by the  
476 finding that Cd influx is affected by Ca<sup>2+</sup> channels blockers (Cohen *et al.*, 1998), as well as by  
477 the correlation between Cd and Ca accumulation in numerous non-metallicolous populations  
478 of *N. caerulea* grown (Sterckeman *et al.*, 2017). The work of Chen *et al.* (2018b) on rice  
479 indicates that the Ca channels OsANN4 and OsGLR3.4 can mediate Cd uptake in roots (Table

480 1). However, their results were obtained after exposing the roots to 20  $\mu\text{M}$  Cd, which is about  
481 1000 times higher than the concentration plants are exposed to in field conditions.

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

## 497 VI. Complexation of Cd by organic ligands in the plant

498 Cadmium can form complexes with organic ligands both in the cytosol and the vacuole. This  
499 reaction is generally considered to be a detoxification process occurring in the roots as well as  
500 in the shoots (Vögeli-Lange and Wagner, 1990; Cobbett and Goldsbrough, 2002; Clemens,  
501 2006; Verbruggen *et al.*, 2009a). In the articles reporting research into this process, it is often  
502 not easy to distinguish the plant organs in which it takes place. This is the reason why, unless  
503 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: ( $\gamma$ -Glu-  
511     Cys)<sub>n</sub>Gly, 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 Cd<sup>2+</sup> 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 Cd<sup>2+</sup> with GSH to form  
526     bisglutathionate-Cd complex, GS<sub>2</sub>-Cd(II). GS<sub>2</sub>-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. caerulescens* 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. caerulescens*,  
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,

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

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

#### 569 **B. Metallothioneins and other cysteine-rich polypeptides**

570 Metallothioneins (MTs) are cysteine rich, low molecular weight, gene-encoded, metal-binding  
571 polypeptides that are ubiquitously found in eukaryotes and cyanobacteria (Cobbett and  
572 Goldsbrough, 2002). It has been shown that MTs play an important role in metal homeostasis  
573 and tolerance in plants (Zimeri *et al.*, 2005; Guo *et al.*, 2008; Ren *et al.*, 2012; Benatti *et al.*,  
574 2014). Elevated expression of MT genes (MT1, MT2, MT3) has frequently been observed in  
575 metal-hyperaccumulating and hypertolerant species or populations (van Hoof *et al.*, 2001;  
576 Mengoni *et al.*, 2003; Roosens *et al.*, 2005; Chiang *et al.*, 2006; van de Mortel *et al.*, 2006;  
577 Hassinen *et al.*, 2007; Jack *et al.*, 2007). However, no positive correlation between these  
578 expression levels and metal tolerance/accumulation was observed. Recently, Peng *et al.*

579 (2017a) proposed that the cysteine-rich MT-like protein SpMTL could act as a cytosolic  
580 chelator to detoxify Cd in the hyperaccumulator *S. plumbizincicola* (Table 1). However, this  
581 role has to be confirmed in other species.

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

### 589 **C. Organic acids**

590 Carboxylic acids also contribute to Cd complexation. Salt *et al.* (1997) showed that during the  
591 initial stages of Cd uptake (6-12 h) by *Brassica juncea* seedlings, most of the intracellular Cd  
592 was bound to oxygen atoms, possibly from organic acids. The spectra of pure Cd citrate fitted  
593 relatively well with the spectra of *B. juncea* seedlings. Later, Ueno *et al.* (2005) showed that  
594 Cd is mainly associated to malate in the leaves of *N. caerulescens*. In roots and shoots of the  
595 hyperaccumulator *Thlaspi praecox*, Vogel-Mikuš *et al.* (2010) found that up to 80% of the Cd  
596 ligands are oxygen ligands provided by the cell walls (see below) and by organic acids stored  
597 in the vacuoles. In the stems and leaves of *Gomphrena claussenii*, a Cd tolerant species, Cd  
598 was mainly bound to oxalate in crystals also containing Ca. The plant was however, exposed  
599 to very high Cd concentrations in hydroponics (100  $\mu$ M CdSO<sub>4</sub>). In the roots, there was no  
600 oxalate crystals and Cd was predominantly associated to S ligands (Pongrac *et al.*, 2018). The  
601 complexation of Cd by organic acids is particularly important in the epidermal cells, where  
602 the vacuoles represent 99% of the symplast volume. In the stems and leaves of the Cd  
603 hyperaccumulating ecotype of *S. alfredii*, Cd is mainly associated with oxygen ligands and the

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

606

## 607 **VII. Storage of Cd in vacuoles of roots and shoots**

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

617 Several transport pathways mediate Cd sequestration in the vacuole either as free Cd or PC-  
618 Cd complexes. They use either ATP hydrolysis or the proton gradient across the vacuolar  
619 membrane as sources of energy to drive substrate accumulation inside the vacuole. Cadmium  
620 can be transported into the vacuole by means of a H<sup>+</sup>/cation-antiporters designated as CAX  
621 (CAtion eXchanger), that use the proton gradient to mediate divalent cation storage in the  
622 vacuole (Salt and Wagner, 1993; Hirschi *et al.*, 2000; Pittman *et al.*, 2004; Shigaki and  
623 Hirschi, 2006; Shen *et al.*, 2012). Among the members of the CAX family that have been  
624 characterized as calcium transporters, CAX1, CAX2 and CAX4 have the ability to transport  
625 Cd as well (Table 1). In *A. thaliana*, CAX2 is strongly expressed in the leaf vascular tissue, in  
626 vegetative meristematic tissue, as well as in the root vascular tissue and in the root tip  
627 (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<sup>2+</sup>, Ca<sup>2+</sup>, Zn<sup>2+</sup>, and Mn<sup>2+</sup> to varying degrees, but that AtCAX4 and AtCAX2 have a  
630 higher Cd<sup>2+</sup> 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<sub>1B</sub>-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

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

704 In contrast with CAX, MTPs, HMA and ABCCs, transporters belonging to the NRAMP  
705 family participate in Cd efflux from the vacuole (Table 1). In *A. thaliana*, AtNRAMP3 and  
706 AtNRAMP4 both reside on the vacuolar membrane (Thomine *et al.*, 2003; Lanquar *et al.*,  
707 2005) and can transport Cd when expressed in yeast (Thomine *et al.*, 2000). Consistent with  
708 its redundant function in Cd efflux from root vacuoles, *nramp3nramp4* double mutant in the  
709 WS accession, which harbours a functional HMA3 gene, accumulates greater Cd  
710 concentrations in roots than the wild type (Chao *et al.*, 2012; Pottier *et al.*, 2015). Conversely,  
711 overexpression of AtNRAMP4 decreases root Cd concentration. In the hyperaccumulator *N.*  
712 *caerulescens*, NcNRAMP3 and NcNRAMP4 have the same protein function as their *A.*  
713 *thaliana* homologues, but are expressed at higher levels (Oomen *et al.*, 2009). The efflux of  
714 Cd and Zn from root vacuoles driven by NcNRAMP3 and 4 may be required for the efficient  
715 translocation of these metals from roots to shoots in *N. caerulescens*.

## 716 VIII. Interactions of Cd with the apoplast

### 717 A. Transport through the apoplast

718 The root apoplast is a compulsory pathway for Cd<sup>2+</sup> and for Cd complexes before entering the  
719 symplast. The solute is considered to move radially in the root apoplast by diffusion and  
720 advection, before reaching the plasma membrane and crossing it through transport proteins  
721 (see above). Cd needs to use the symplastic route to cross the exodermis and/or the  
722 endodermis cell layers, where apoplastic transport is blocked by the apoplastic barriers  
723 (Casparian strip, suberine lamellae, secondary thickening). It has however been suggested that  
724 Cd could reach the xylem *via* the sole apoplastic pathway in the parts of the root where the  
725 apoplastic barriers are lacking or incomplete, namely the root tip, stage I endodermis and sites

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

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

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

#### 744 ***B. Storage in the apoplast***

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

776 2010). Li *et al.* (2015) showed that more Cd was retained in the root cell wall of the non-  
777 hyperaccumulating ecotype (NHE) of *S. alfredii* than in the hyperaccumulating one (HE).  
778 Transcriptomic analyses also indicated an important role played by the cell wall, in the Cd  
779 hyperaccumulation in *S. plumbizincicola* (Peng *et al.*, 2017b). As in *A. thaliana*, Cd treatment  
780 induced an increase in cell-wall polysaccharides (pectins and hemicelluloses) in the NHE of  
781 *S. alfredii*, but not in the HE. Consequently, more Cd was available for xylem loading in the  
782 HE ecotype.

783 It should be noted that in several of the above-cited works (Lozano-Rodriguez *et al.*, 1997;  
784 Carrier *et al.*, 2003; Cao *et al.*, 2018a), as well as in others (e.g. (Fu *et al.*, 2011; Wang *et al.*,  
785 2015a; Wang *et al.*, 2015b), the method used to determine Cd (or other elements) in cell walls  
786 is questionable. Indeed, it is based on a fine grinding of the fresh tissues in a buffer solution to  
787 destroy the cells. The homogenate is generally then sieved to collect the cell walls. The filtrate  
788 is centrifuged to separate chloroplasts from other organelles and from cytosolic and vacuole  
789 solution. This procedure in liquid phase does not exclude the diffusion of Cd from one  
790 fraction to another during the tissue grinding and fraction separation. The content measured in  
791 each fraction might not therefore, correspond to the distribution of the metal *in vivo*.

## 792 **IX. Translocation and reallocation of Cd**

793 Cadmium concentration is often higher in the roots than in the shoots (Jarvis *et al.*, 1976;  
794 Lozano-Rodriguez *et al.*, 1997; Harris and Taylor, 2013; Cornu *et al.*, 2016), and generally  
795 lower in the seeds than in the stem and leaves (Hinesly *et al.*, 1978; Wiggenhauser *et al.*,  
796 2016; Chen *et al.*, 2018a). In some species, such as lettuce (*Lactuca sativa*), spinach (*Spinacia*  
797 *oleracea*), cauliflower (*Brassica oleracea*) and oat, concentrations are higher in the shoot than  
798 in the roots when plants are grown on low Cd contaminated soils. However, when the plants  
799 are grown on more contaminated soil, the root concentration becomes higher than that of the  
800 shoots (John, 1973). In maize, Perriguet *et al.* (2008) observed increasingly greater

801 concentrations in roots than in shoots and an increase in the Cd root-to-shoot ratio, as that of  
802 the Cd concentration in the nutrient solution rose. The ratio sharply decreased after reaching a  
803 peak, as if the root retention capacities became overloaded at this point. The sequestration of  
804 Cd in the roots up to a certain limit corresponds to the excluder strategy proposed by Baker  
805 (1981) for certain species. This author also defined an accumulator strategy, “where metals  
806 are concentrated in above-ground plant parts from low or high soil levels”. This is the case for  
807 *N. caerulescens*, *S. nigrum* or *S. alfredii* (Yang *et al.*, 2004; Wei *et al.*, 2006; Lovy *et al.*,  
808 2013), which can be regarded as Cd hyperaccumulators as the Cd concentration in their shoots  
809 can reach 100 mg Cd (kg DW)<sup>-1</sup> (van der Ent *et al.*, 2013).

#### 810 **A. Cd loading into the xylem**

811 HMA transporters play an important role in the loading of Cd in xylem vessels. As ATPases,  
812 they can transport the cation against its electrochemical gradient, from the cytosol into the  
813 xylem sap. AtHMA4 is expressed at its highest level in tissues surrounding the root vascular  
814 vessels in *A. thaliana* (Mills *et al.*, 2003; Verret *et al.*, 2004). Located in the plasma  
815 membrane and acting as an efflux pump, HMA4 plays a role in metal loading into the xylem,  
816 thereby enhancing the root-to-shoot translocation of Zn and Cd in *A. thaliana* (Verret *et al.*,  
817 2004; Mills *et al.*, 2005). Together with AtHMA4, its paralogue AtHMA2 was shown to be  
818 involved in the root-to-shoot transfer of Cd (Wong and Cobbett, 2009; Wong *et al.*, 2009)  
819 (Table 1). Moreover HMA4 is also important for Cd translocation in the hyperaccumulator *N.*  
820 *caerulescens* (Papoyan and Kochian, 2004). Accordingly, in *A. halleri*, enhanced expression  
821 and a triplication of HMA4 was shown to be responsible for the hypertolerance to Cd  
822 (Courbot *et al.*, 2007; Hanikenne *et al.*, 2008). In *N. caerulescens*, the HMA4 gene is also  
823 present as multiple tandem copies (Ó Lochlainn *et al.*, 2011) and differences in HMA4  
824 expression correspond to the differences in Cd translocation and accumulation among  
825 different populations of *N. caerulescens* (Craciun *et al.*, 2012).

826 In rice, a single gene, OsHMA2, which is orthologous to AtHMA2 and AtHMA4, encodes an  
827 efflux transporter involved in Cd xylem loading. OsHMA2 is constitutively expressed in the  
828 vascular bundles of roots at the vegetative stage, and knocking-out this gene results in lower  
829 root-to-shoot translocation and grain content of Cd (Nocito *et al.*, 2011; Satoh-Nagasawa *et*  
830 *al.*, 2012; Takahashi *et al.*, 2012; Fontanili *et al.*, 2016). OsHMA2 is also expressed in the  
831 phloem tissue of nodes (Yamaji *et al.*, 2013). To account for the role of OsHMA2 in the  
832 control of Cd transfer between phloem and xylem in nodes, Yamaji *et al.* (2013) proposed that  
833 OsHMA2 could function as an influx transporter for both Cd and Zn. Such reverse transport is  
834 extremely unlikely, as the function of HMAs in metal efflux has been conserved across  
835 kingdoms and the transport mechanism involves ATP hydrolysis at the cytosolic side of the  
836 protein. Based on a Cd/Zn reciprocal competition study, Fontanili *et al.* (2016) suggested that,  
837 beside OsHMA2, another transport system which is not affected by the presence of  $Zn^{2+}$ , yet  
838 to be identified, may mediate root-to-shoot Cd translocation in rice. Recently, Hao *et al.*  
839 (2018) suggested that OsCCX2, a putative cation/calcium (Ca) exchanger mainly expressed in  
840 xylem region of the nodes, could be an efflux transporter, controlling Cd loading into xylem  
841 (Table 1).

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

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

### 852 **B. Transport in the xylem**

853 Once loaded into the root xylem, Cd migrates to the aerial parts with the xylem sap flow,  
854 driven by plant transpiration, *i.e.* by the gradient of water potential between the soil and the  
855 atmosphere. Numerous works show that Cd concentration in aerial plant parts increases with  
856 the concentration in the root exposure solution (see for instance Jarvis *et al.* (1976); Florijn  
857 and Van Beusichem (1993a); Lovy *et al.* (2013)). Moreover, various investigations have  
858 shown that Cd content in the xylem sap increases with Cd content in the exposure solution  
859 (Salt *et al.*, 1995; Wei *et al.*, 2007; Ueno *et al.*, 2008; Nakamura *et al.*, 2013; Hazama *et al.*,  
860 2015; Cornu *et al.*, 2016).

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

### 869 **C. Cadmium speciation in the xylem sap**

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

874 White *et al.* (1981) have shown that Cd forms negatively-charged complexes in soybean and  
875 tomato xylem sap. In soybean, Cataldo *et al.* (1988) found Cd as a single, slightly anionic  
876 species and as a cationic component, which appeared to be inorganic Cd. Citric acid promotes  
877 the translocation of Cd in tomato, which may be due to the formation of citrate Cd complexes  
878 in the xylem (Senden *et al.*, 1995). In contrast, in the xylem sap of *B. juncea*, Salt *et al.* (1995)  
879 found that Cd is coordinated either with oxygen or with nitrogen, which excludes a  
880 complexation by PCs or MTs. Based on the analysis of low molecular weight compounds in  
881 the xylem sap of *B. juncea*, Wei *et al.* (2007) calculated that the Cd speciation was 33%  
882 organic acid–Cd and 67% inorganic anion–Cd in plants exposed to a low Cd concentration  
883 ( $10^{-8}$  M). In the xylem of plants exposed to 10  $\mu$ M Cd, the calculated Cd speciation was 1%  
884 PC–Cd, 35% organic acid–Cd, and 64% inorganic anion–Cd.

885 According to Ueno *et al.* (2008), based on nuclear magnetic resonance measurement and  
886 modelling of chemical speciation, Cd is essentially present as a free ion in the xylem sap of  
887 the hyperaccumulator *A. halleri*. In castor bean plants (*Ricinus communis*) exposed to 1  $\mu$ M  
888 Cd for 3 weeks, 92% of the Cd in xylem was found to be present as a free cation (Hazama *et*  
889 *al.*, 2015). In sunflower exposed to low and realistic Cd concentrations (2.5 nM or 20 nM),  
890 Cornu *et al.* (2016) determined that the fraction of free Cd ion in the xylem sap was around  
891 50%. These results are difficult to reconcile, as they suggest that depending on the species and  
892 on the Cd concentration in the medium, Cd may be transported within the xylem both as  
893 complexes with organic and inorganic acids, but also as a hydrated free cation.

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

895 After their long-distance transport in the xylem sap towards the aerial parts of the plant,  
896 mineral ions are unloaded from the xylem vessels to be distributed in the other leaf tissues  
897 (Figure 5). Xylem unloading of Cd is expected to follow the same process as the nutrient ions.  
898 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<sup>2+</sup>  
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. caerulea* (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  $^{107}\text{Cd}$  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*

1022 *al.* (2010) showed that, in rice phloem sap, Cd may bind to a 13 kDa protein distinct from  
1023 MT. Cd is also associated to SH compounds, probably PCs and GSH. Hazama *et al.* (2015)  
1024 studied the speciation of Cd in the phloem sap of the castor bean (*R. communis*). Based on  
1025 size-exclusion HPLC, they found that about 50% of the metal is bound to Cd-containing  
1026 proteins or peptides, mixtures of proteins and PCs (such as PC4 and PC3) as well as  
1027 complexes with GSH and PC2.

## 1028 **X. Interaction of Cd with other elements**

1029 Cadmium absorption by roots can be affected by the other elements in the soil solution, in  
1030 three ways. First, as mentioned above, some anions such as  $\text{Cl}^-$ ,  $\text{NO}_3^-$ , or  $\text{SO}_4^-$ , can form  
1031 complexes with Cd and modify the diffusion and the absorption of the metal. Moreover,  
1032 major ions such as  $\text{H}^+$ ,  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ , present at concentrations several orders of magnitude  
1033 higher than those of Cd, decrease the plasma membrane surface potential, by compensating  
1034 the negatively-charged lipid heads present at the external side of the plasma membrane. In the  
1035 absence of negative charges, which would attract them, the local concentrations of trace  
1036 cations, such as  $\text{Cd}^{2+}$ , at the vicinity of the plasma membrane are reduced. The lower local  
1037 concentration around the uptake transporters results in lower uptake (Kinraide, 1998; Wang *et*  
1038 *al.*, 2011; Wang *et al.*, 2012). On the other hand,  $\text{Cd}^{2+}$  and other cations directly compete for  
1039 transport. Using soybean in hydroponics, Cataldo *et al.* (1983) showed that root absorption of  
1040  $\text{Cd}^{2+}$  fell in the presence of  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Mn}^{2+}$ , and  $\text{Zn}^{2+}$ , thus suggesting that these cations  
1041 compete with  $\text{Cd}^{2+}$  to enter the root through the same transport systems. Hart *et al.* (2002)  
1042 also observed that trace amounts of Zn in the exposure solution inhibit Cd uptake by roots of  
1043 durum wheat (and vice versa), thereby indicating that the two ions compete for root uptake  
1044 via the same transport systems. In contrast, Salt *et al.* (1997) found no competition of  $\text{Mn}^{2+}$   
1045 and  $\text{Zn}^{2+}$  with  $\text{Cd}^{2+}$  for root absorption by *B. juncea*. Sterckeman *et al.* (2011) exposed maize

1046 root plants to a 1  $\mu\text{M}$  Cd solution with 5  $\mu\text{M}$  of another cation ( $\text{Ca}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Mg}^{2+}$ ,  
1047  $\text{Mn}^{2+}$ ,  $\text{Na}^+$ ,  $\text{NH}_4^+$ ,  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$  or  $\text{Zn}^{2+}$ ) and found a reduction in Cd uptake by  $\text{Cu}^{2+}$  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. caerulescens* 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  $\text{Fe}^{2+}$  prevents Cd uptake by inhibiting IRT1  
1064 expression and favouring  $\text{Fe}^{2+}$  in its competition with  $\text{Cd}^{2+}$ . 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. caerulea* with  $\text{NO}_3^-$   
1087 resulted in a doubling of the Cd concentration in the shoots compared with  $\text{NH}_4^+$  supply.  
1088 Moreover, when cultivating the same species in soil, Cd uptake was highest in the  $\text{NO}_3^-$   
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  $\text{NO}_3^-$  on Cd absorption are still unclear. It  
1094 has been proposed that the uptake of  $\text{NO}_3^-$  would favour Cd uptake by increasing membrane

1095 potential, while that of  $\text{NH}_4^+$  would depolarize the membrane (Monsant *et al.*, 2010). More  
1096 recently, based on results obtained with rice, Yang *et al.* (2016) proposed that  $\text{NO}_3^-$  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  $\text{NH}_4^+$  than with  
1099  $\text{NO}_3^-$  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 SiO<sub>3</sub> 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,

1144 such as calcium, zinc, manganese or iron to accumulate in plants. Moreover, the pathways  
1145 used by Cd differ according to plant species. Investigations in the model species *A. thaliana*  
1146 and in rice have revealed striking differences in the uptake mechanisms, as well as  
1147 commonalities in the mechanisms that allow Cd sequestration in roots. There will be a need to  
1148 extend this knowledge to other species, including species with contrasting accumulations and  
1149 crops such as lettuce, cocoa and tobacco, for which Cd accumulation is an important public  
1150 health issue. Moreover, the precise location of root Cd carriers at the tissue scale, will need to  
1151 be determined in the future in order to better describe Cd uptake paths into plants.

1152 Among crop species, rice has been the subject of recent major advances. Whilst rice produces  
1153 the staple diet of hundreds of millions of people in the world, it is the cereal that accumulates  
1154 Cd at the highest level in its grains. These scientific advances are about to lead to the release  
1155 of rice cultivars that accumulate much less Cd. In contrast, few results have been obtained on  
1156 bread wheat (*T. aestivum*) and durum wheat, whereas these cereals are responsible for the  
1157 chronic exposure to Cd of a very large portion of the world population (EFSA, 2012). In the  
1158 future, research should focus on the biomolecular mechanisms of Cd accumulation in wheat.

1159 Recent works have suggested the involvement of new actors in Cd transport in plants, such as  
1160 CAL1, a defensin-like protein, which would appear to chelate the metal and favour its xylem  
1161 loading, or again the homeodomain-containing protein gene, OsHB4a, which regulates several  
1162 genes involved in Cd uptake and translocation. The regulation of OsHB4a by the miR166 also  
1163 indicates that other mechanisms involving micro-RNA may be important for the movement of  
1164 Cd in a plant. The effects of Si or of N forms on Cd accumulation are relatively new  
1165 discoveries. These correspond to complex processes, which are as yet only partly explained,  
1166 but may help to improve the management of Cd-contaminated land. All these topics represent  
1167 avenues of research leading to the possibility of reducing Cd accumulation in crops.

1168

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1172

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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<sub>s</sub>: Organic ligand originating from the soil; L<sub>p</sub>: 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 <sup>2+</sup> , Fe <sup>2+</sup> , Fe <sup>3+</sup> , Mn <sup>2+</sup> , Zn <sup>2+</sup>	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 <sup>2+</sup>	Fe deficiency	Nakanishi <i>et al.</i> (2006)
	ZNT1		Roots, shoot	<i>Noccaea caerulea</i>	Zn <sup>2+</sup>	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> <sup>1</sup> , <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 <sup>2+</sup>		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> <sup>1</sup> , <i>H. vulgare</i>			Tommasini <i>et al.</i> (1998); Schneider <i>et al.</i> (2009)
	MRP7		Roots, shoot	<i>A. thaliana</i> <sup>2</sup>			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> <sup>2</sup>	Ca <sup>2+</sup> , Zn <sup>2+</sup> , Mn <sup>2+</sup>		Pittman <i>et al.</i> (2004); Koren'kov <i>et al.</i> (2007)
	CAX4		Roots, shoot	<i>A. thaliana</i> <sup>2</sup>	Ca <sup>2+</sup> , Zn <sup>2+</sup> , Mn <sup>2+</sup>		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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2335 **Figure 1.** Principal reactions and processes controlling cadmium availability at the root-soil interface. PM: Plasma membrane; L<sub>s</sub>: Organic  
 2336 ligand originating from the soil; L<sub>p</sub>: organic ligand originating from rhizodeposition. For simplification, the cell wall is not represented.

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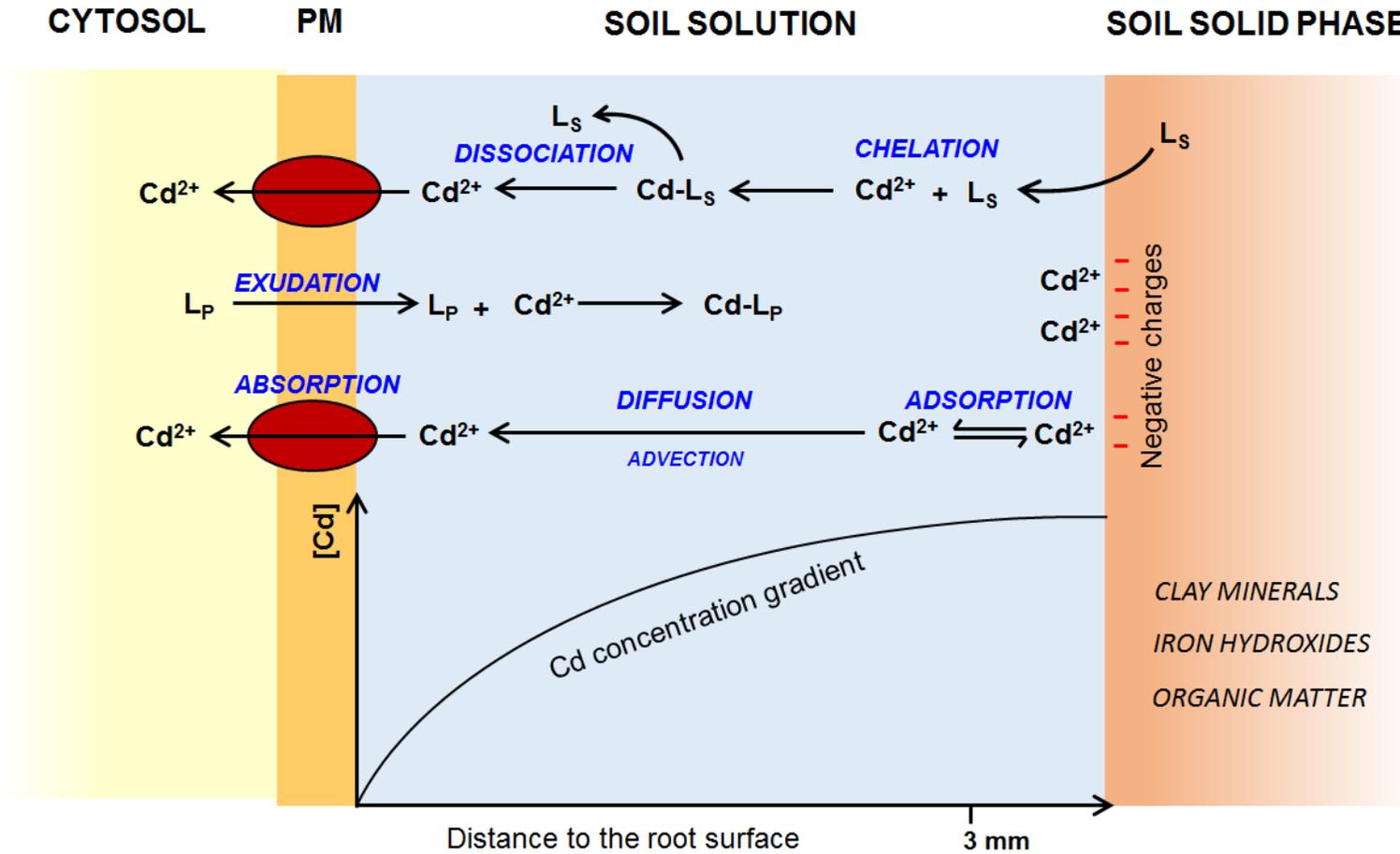
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2348 **Figure 2.** Schematic representation of *Arabidopsis thaliana* root with the gene/proteins involved in Cd transport. For simplification, the cell wall  
 2349 is not represented.

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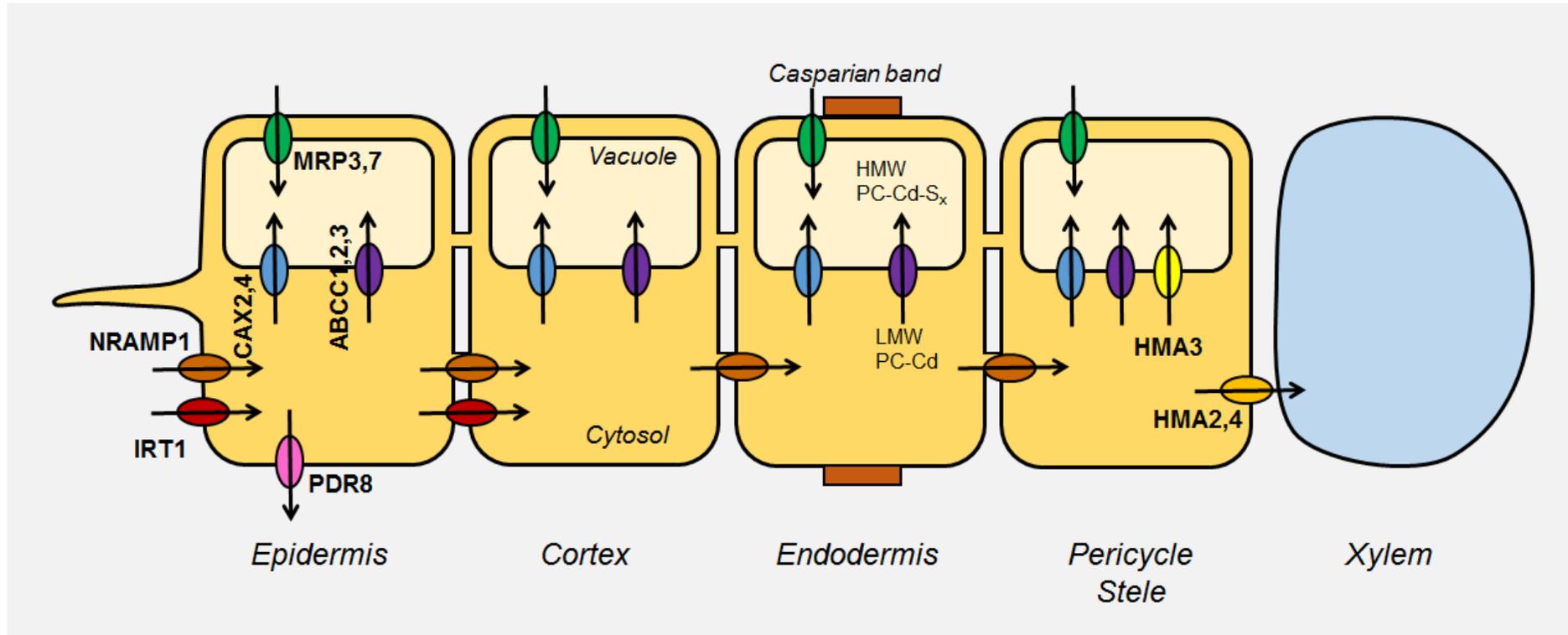
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2360 **Figure 3.** Schematic representation of a rice (*Oryza sativa*) root with the gene/proteins involved in Cd transport. For simplification, the cell wall is not represented. A question mark after a protein name means it might not be expressed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

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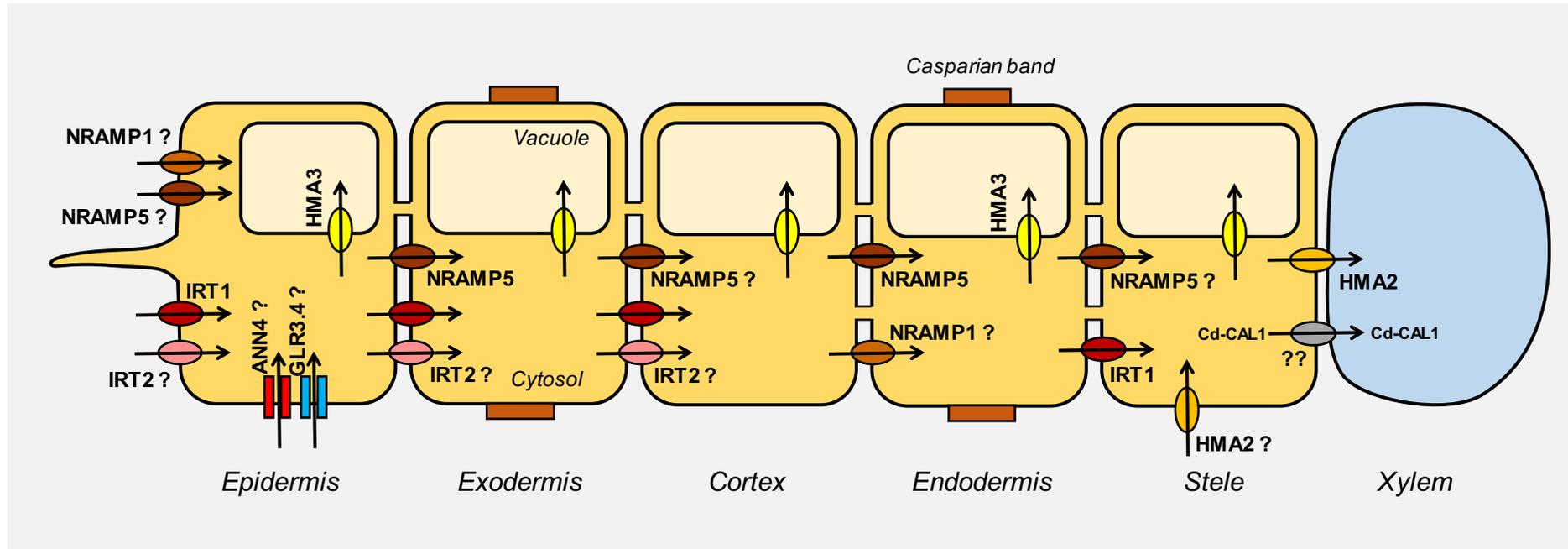
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2372 **Figure 4.** Schematic representation of the hyperaccumulator *Noccaea caerulescens* root with the gene/proteins involved in Cd transport. For  
 2373 simplification, the cell wall is not represented.

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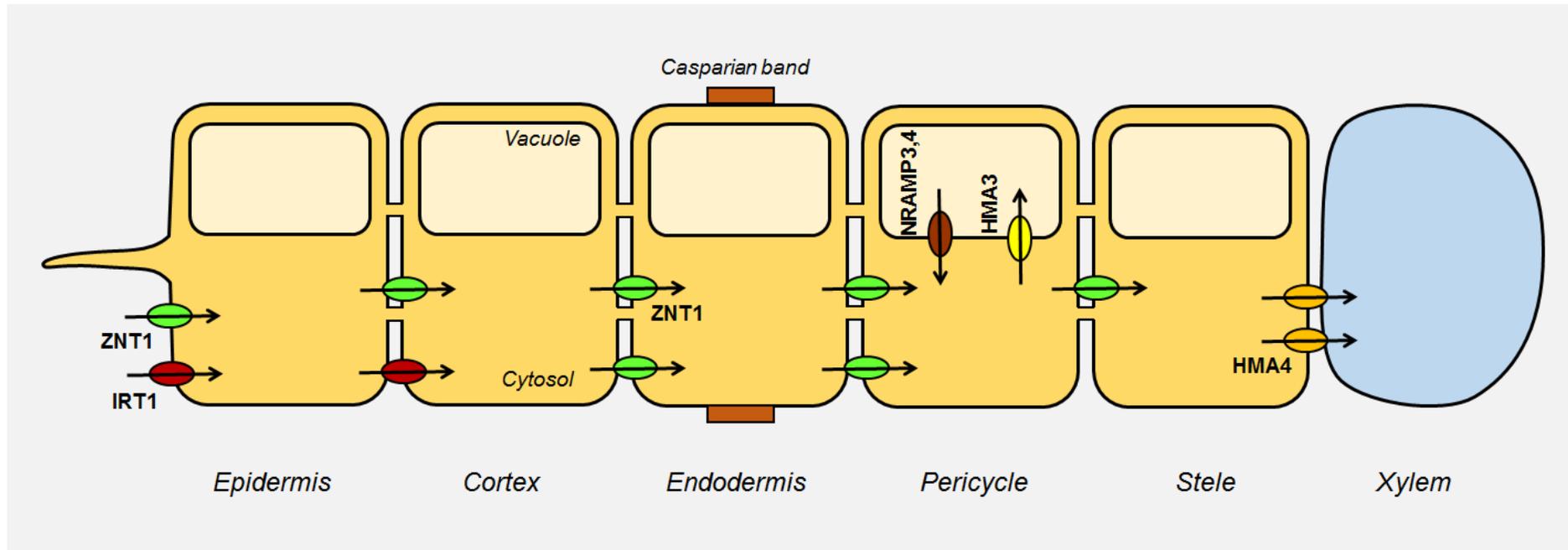
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2383 **Figure 5.** Schematic representation of leaf tissues with the transporters involved in Cd distribution. The prefix before the gene name indicates the  
 2384 plant species in which it was observed. Nc: *Noccaea caerulescens*, Os: *Oryza sativa*. 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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