Review

Heavy Metals in Crop Plants: Transport and Redistribution Processes on the Whole Plant Level

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Abstract: Copper, zinc, manganese, iron, nickel and molybdenum are essential micronutrients for plants. However, when present in excess they may damage the plant or decrease the quality of harvested plant products. Some other heavy metals such as cadmium, lead or mercury are not needed by plants and represent pollutants. The uptake into the roots, the loading into the xylem, the acropetal transport to the shoot with the transpiration stream and the further redistribution in the phloem are crucial for the distribution in aerial plant parts. This review is focused on long-distance transport of heavy metals via xylem and phloem and on interactions between the two transport systems. Phloem transport is the basis for the redistribution within the shoot and for the accumulation in fruits and seeds. Solutes may be transferred from the xylem to the phloem (e.g., in the small bundles in stems of cereals, in minor leaf veins). Nickel is highly phloem-mobile and directed to expanding plant parts. Zinc and to a lesser degree also cadmium are also mobile in the phloem and accumulate in meristems (root tips, shoot apex, axillary buds). Iron and manganese are characterized by poor phloem mobility and are retained in older leaves.

Keywords: heavy metals; micronutrients; pollutants; transport; xylem; phloem; redistribution
1. Heavy Metals: Micronutrients or Pollutants?

Several heavy metals (e.g., Fe, Mn, Cu, Zn, Ni, and Mo) are required in traces as micronutrients by plants [1–3], while others (e.g., Cd, Cr, Hg, and Pb) are known as pollutants [4–12]. Major functions of heavy metals in plant metabolism are based on their involvement in oxidation/reduction processes [1,2,13]. Fe is essential as central ion in heme proteins (e.g., in cytochromes, nitrate reductase, catalase, and peroxidase), in siroheme proteins (e.g., nitrite reductase and sulfite reductase), in iron-sulfur proteins (e.g., ferredoxin) and in other iron-containing proteins (e.g., lipoxygenases) [1,2,13–16]. Ferritin is located in plastids and represents important intracellular storage form for Fe [17–19]. Mn is essential for the oxygen evolution in photosystem II and for a series of enzymatic reactions (e.g., phosphoenolpyruvate carboxykinase, and superoxide dismutase) [1,2,13,20–22]. Cu is present in the plastidial plastocyanin, in the mitochondrial cytochrome c oxidase, in Cu-Zn superoxide dismutase as well as in a series of other proteins [1,2,13,23–26]. Zn is essential for several enzymes (e.g., metalloproteinase, carbonic anhydrase, and Cu-Zn superoxide dismutase) [1,2,13,26–30]. Ni is known for its involvement in urease activity [1,2,13,14,31–34]. Mo (present in the soil solution as molybdate anion) is part of the molybdenum cofactor, which is required for nitrate reductase or xanthine dehydrogenase activity [1,2,13,14,35–37]. Co is an interesting element in this context, since it is not required by the higher plant itself, but is essential for the microorganisms involved in symbiotic nitrogen fixation [2,38,39]. Even those heavy metals required as micronutrients by all plants may become pollutants at elevated levels [2,40,41]. This review is focused on long-distance transport of heavy metals in crop plants, since the redistribution via xylem and phloem is important for the micronutrient supply of various plant parts and also for the accumulation of undesirably large quantities damaging the plant or negatively influencing the quality of harvested plant products.

The importance of heavy metal homeostasis in plants is illustrated in Figure 1. Heavy metals present at elevated levels may cause an increased production of reactive oxygen species (ROS) in plant cells on one hand [42–51] and may be involved in the enzymatic detoxification of ROS on the other hand [2,3,42,52–54]. Stresses (e.g., drought, heat and high light intensity) may also cause an accumulation of ROS and of ROS-damaged cell constituents [54,55]. These findings underline the importance of heavy metal homeostasis for plant cells. Several forms of superoxide dismutases are present in plants: Cu/Zn superoxide dismutases (usually present under several forms) were reported to be present in various subcellular compartments, while Mn superoxide dismutase isoforms were localized in the mitochondria and Fe superoxide dismutase was reported to be a plastidial enzyme [1,2,54]. Especially Mn superoxide dismutase activity was found to be increased under drought stress [54].
Figure 1. Involvement of micronutrients (e.g., Fe, Mn, Zn, Cu and Ni) in the generation and detoxification of ROS in plant cells under abiotic and biotic stress. Micronutrient deficiency and excess influence ROS levels and as a consequence damages caused by them.

2. Transport with the Transpiration Stream in the Xylem

Numerous processes in the soil, which are not subject of this review, may influence heavy metal solubility and as a consequence the availability for plants [2]. In some cases, heavy metals (e.g., Co, Cr, and Fe) are retained in the roots and only a minor portion reaches the shoot [56–61]. This retention can be due to insolubilization (e.g., at the root surface and in the root apoplast) [56,57] or to a compartmentation in cells avoiding the release to the xylem [58,59]. Oxygen released from the roots may cause oxidation and insolubilization of iron in the root apoplast of rice grown in waterlogged soil [57].

A strong retention in the roots was detected in wheat and lupin for Co [60,61] and in lupin also for Cd [60]. These elements were present in soluble form, but only a minor portion reached the vascular cylinder suggesting that compartmentation played a major role [58–60]. The abundance of ligands and the formation of heavy metal complexes with organic acids [62,63], with phytochelatin [64] or with nicotianamine [65] were found to be important for the retention in the roots. Good evidence was presented for the involvement of Ni-histidine complexes in the vacuolar compartmentation of Ni and as a consequence in the retention of this heavy metal in roots [59]. A heavy metal ATPase was suggested to be involved in Cd accumulation in vacuoles of root cells causing Cd retention in roots and decreasing the transport to the shoot [66].

Heavy metals are transported with the transpiration stream in the xylem from the roots to transpiring shoot parts (e.g., photosynthesizing leaves) [60,61,67]. After the release into the root xylem, free or chelated ions flow with the xylem sap upwards (Figure 2). Important for the concentration of heavy metals in the transpiration stream are xylem loading in the roots, interactions with cell walls during acropetal transport and selective removal from the xylem sap [65,66,68,69]. If there would be no further redistribution, the heavy metals would accumulate primarily in photosynthetically active (transpiring) leaves. Such an accumulation with no or only a minor redistribution was observed for the micronutrient Mn as well as for the macronutrient Ca and to a minor extent for Fe (Figures 2 and 3) [2,67–70]. Although
the xylem vessels are dead, membranes of living cells around the vessels allow a selective removal of ions from the xylem sap [68,69].

**Figure 2.** Xylem and phloem transport in the intact plant. Zn, Ni and Mn are readily transported via xylem to the shoot. Mn is essentially immobile in phloem, while Ni is rapidly redistributed to the youngest (expanding) plant parts and Zn is more slowly redistributed via the phloem (accumulation in meristems, but also well mobile in the phloem).
Figure 3. Autoradiographs documenting $^{65}$Zn and $^{59}$Fe distribution and redistribution in millet, mustard, tomato and dwarf bean. Young plants were labeled for one day with nutrient solution containing $^{65}$Zn or $^{59}$Fe. The roots of the labeled plants were rinsed several times with unlabeled nutrient solution and then incubated for up to 12 days (bean), 15 days (mustard, tomato) or 21 days (millet). Plants collected throughout the incubation period were dried, fixed on cardboard and then exposed in darkness to an X-ray film. The relative distribution of the label in a plant must be considered and not the absolute label content in a particular plant part, since initial labeling was not identical.

3. Redistribution via the Phloem

The symplastic transport via the phloem allows a redistribution of nutrients, assimilates and pollutants within the plants and depends on the actual source/sink network (Figure 2) [71–73]. Phloem mobility of heavy metals varies in a wide range. Ni is highly phloem-mobile and can be repeatedly redistributed in crop plants throughout vegetative growth and the reproductive phase (Figure 2) [60,61,69]. Zn is also mobile in plants and can be transported via the phloem to growing plant parts (Figure 2) [60,61,68,69]. Cd (a pollutant and not a nutrient for plants) is less phloem-mobile than Zn, but it is also to some extent redistributed via the phloem [60,61,69]. An extremely poor redistribution via the phloem was found for Mn and Fe [60,61,69]. Although these two elements are micronutrients, they are not easily transported from mature organs to major sinks (e.g., developing roots, emerging leaves, maturing fruits). Distribution patterns for several heavy metals are summarized in Figure 4. Approaches to identify redistribution processes via the phloem include balance sheets for contents in various organs [60,61,70,74], introduction of radiolabeled heavy metals into a defined leaf via a flap [69] and phloem interruption (e.g., by steam-girdling) [68,69,74].

Source strength and sink strength are important for the direction and the velocity of phloem sap flow, while phloem loading is essential for the transported compounds [71–73]. The delivery through plasmodesmata in symplastic phloem loaders and the uptake through the membrane of the sieve tube-companion cell complex are crucial for the composition of the phloem sap [71–73]. Within the shoot, heavy metals can be redistributed from senescing leaves via the phloem to sinks (e.g., growing vegetative parts and maturing fruits). Another possibility is the transfer to the phloem before the xylem sap reaches mesophyll cells [2,68,69]. Several types of metal-binding compounds including nicotianamine and phytochelatins were reported to be relevant for the transport of heavy metals in the phloem [75–77]. A transporter for the copper–nicotianamine complex was found to be located in the phloem of rice leaves and was proposed to be important for the translocation of Cu from leaves to developing organs and maturing seeds [78].
Figure 4. Scheme summarizing the processes involved in the distribution and redistribution of heavy metals in plants. The relative distribution patterns (red color) of $^{65}$Zn, $^{109}$Cd, $^{63}$Ni, $^{59}$Fe, $^{54}$Mn and $^{57}$Co are based on autoradiographs with various plant species emphasizing important findings. The following processes are involved in generating these radionuclide-specific distribution patterns: insolubilization at the roots surface and in the root apoplast (A); uptake and loading into the root xylem (B); uptake and retention in root cells (C); basipetal transport to the roots via the phloem (D); cell-to-cell transport in meristems and regions without functional phloem (E); transport with the transpiration stream in the xylem to mesophyll cells (F); transfer from xylem to phloem via transfer cells in small bundles (G); and remobilization from senescing leaves and transport to sinks via the phloem (H).
The redistribution from expanded leaves to sinks represents besides the flux from the roots to the shoot an additional control mechanism for the heavy metal contents in emerging organs and maturing fruits and seeds [68,70,74,79]. Fe and Mn are reduced in waterlogged soil to the well water-soluble Fe$^{++}$ and Mn$^{++}$ ions increasing for these two elements the concentration in the soil solution [70]. As a consequence, higher Fe and Mn contents can be detected in wheat shoots under such conditions [70]. The contents are drastically increased in leaves and glumes, while the grains are far less affected [70]. These findings indicate that the control of redistribution processes via the phloem is important for the composition of harvested grains [70]. Elevated concentrations of Zn, Ni, Co or Cd cause somewhat increased contents in wheat grains, but leaves and glumes are far more affected suggesting again a control of heavy metal delivery to the grains via the phloem [68,80,81].

4. Xylem/Phloem Interactions

Xylem and phloem are two different long-distance transport systems with different properties. Functional xylem and phloem are often separated by a cambium and not yet fully differentiated elements (e.g., in stems of dicotyledonous plants or in gymnosperms) [82]. Rays may allow a xylem–phloem exchange of solutes [84]. Functional xylem and phloem elements are close together and may interact more directly in the small stem bundles of cereals or in minor leaf veins without cambium and without formation of secondary vascular tissues [82,83]. This arrangement allows the selective transfer of solutes from the xylem to the phloem [2,84]. Transfer cells with a proliferated cell surface are presumably key players for the selective xylem-to-phloem transfer of heavy metals and other solutes (Figure 4) [2,84,85]. From steam-girdling experiments and heavy metal balance sheets it became evident that in the stem of wheat some heavy metals (e.g., the micronutrients Zn and Ni and the pollutant Cd) are transferred from the xylem to the phloem, while other heavy metals (e.g., the micronutrient Mn or Fe) are not or only much less efficiently loaded into the phloem [68,69,74,80]. Since the transfer of $^{65}$Zn from the xylem to the phloem was also observed in the wheat peduncle without node, it can be concluded that this type of phloem loading occurs in the small bundles in internodes and that the node is not a prerequisite [68]. Such a selective xylem-to-phloem transfer (e.g., in the peduncle of wheat or in minor veins of lupin) allows a channelling of solutes to the maturing grains/seeds. In leaves of legumes labeled via the roots for a short period (e.g., for one day) and then incubated for several days in unlabeled medium clear differences between the labeling patterns with $^{65}$Zn and $^{59}$Fe can be observed (Figures 3 and 4). The veins in bean leaves are strongly labeled after $^{65}$Zn introduction, while other leaf cells contain far less $^{65}$Zn. This pattern can be explained by the transfer from the rapidly flowing xylem to the more slowly flowing phloem (Figure 4). In contrast, $^{59}$Fe is mainly present in interveinal regions, while the veins are far less labeled (Figures 3 and 4). Such a labeling pattern can be explained by the flux with the transpiration stream to minor leaf veins followed by an accumulation in non-vascular tissues (Figure 4), as was proposed previously for some amino acids [84].

Some heavy metals (e.g., Ni, Zn, Co, and Cd) may be delivered to roots by basipetal transport in the phloem (Figure 4) [69]. Afterwards they may be partially loaded again into the xylem and flow upwards with the transpiration stream. $^{63}$Ni, $^{65}$Zn and $^{109}$Cd are redistributed via the phloem and can be translocated to previously unlabeled roots and to newly formed leaves [60,61]. However, the distribution of $^{63}$Ni differs from that of $^{65}$Zn and $^{109}$Cd [60,61,86]. While $^{63}$Ni accumulates in expanding leaves and
in root parts behind the meristem, $^{65}\text{Zn}$ and $^{109}\text{Cd}$ strongly accumulate in root tips, apical shoot meristems and axillary buds [60,61,86]. This difference might be caused by a transport of $^{63}\text{Ni}$, $^{65}\text{Zn}$ and $^{109}\text{Cd}$ via the phloem followed by an efficient cell-to-cell transport of $^{65}\text{Zn}$ and $^{109}\text{Cd}$ (but not of $^{63}\text{Ni}$) to the meristem through the not yet fully differentiated regions without a functional phloem [60,61,86].

5. Selective Accumulation of Heavy Metals in Harvested Plant Parts

The contents of heavy metals in plant products for human nutrition or in fodder plants are important for the quality of the harvest [87–98]. It must be distinguished between seed/grain crops such as cereals or soybean and plants of which the whole shoot is harvested such as plants used for silage or grasslands. Good phloem mobility is a prerequisite for the redistribution of heavy metals from leaves to maturing seeds. When whole shoots are used for human or animal nutrition, the total content in the shoot is very important, while redistribution via the phloem is far less relevant. In contrast, phloem transport is highly relevant when only cereal grains or seeds of dicotyledenous plants are harvested.

In certain regions a sufficient supply of heavy metals for humans (e.g., Fe and Zn) is an issue [87–91]. The uptake of heavy metals into the plants, the transport to the shoot and redistribution processes within the shoot are involved in controlling the contents in harvested products. Breeding is a key aspect in the context of biofortification of plant products with heavy metals to overcome deficiencies in humans and animals [88,90]. Furthermore, negative effects of antinutrients interfering with heavy metal availability (e.g., phytate, oxalate) must be considered when evaluating the bioavailability of essential heavy metals [90,91].

Elevated contents of heavy metals—especially of pollutants such as Cd, Cr or Pb—can also decrease the quality of harvested plant products [92–98]. Undesirably high or even toxic levels are a rather local, but nevertheless an important problem. Low uptake rates for the pollutant(s), a slow root-to-shoot transfer and a minimized redistribution to harvested shoot parts is desirable for crops grown on polluted soil. Contaminated irrigation water, the use of sewage sludge for fertilization, the release of pollutants from industrial processes and an improved solubilization of some heavy metals under extreme climatic conditions (e.g., flooding) may cause undesirably high concentrations in plant products [93–98]. Even when the input of heavy metals in contaminated soil is drastically lowered, fields may still contain high heavy metal contents for decades. A cautious selection of crop species and varieties, optimized nutrient supply, and phytoremediation of the field may contribute to improve the situation.

6. Relevance of Long-Distance Transport for Phytoremediation

Hyperaccumulators of heavy metals are important for phytoremediation of polluted soils [99–101]. Such plants should have properties clearly differing from desirable properties of crop plants. Hyperaccumulators should efficiently take up heavy metals from the soil, should transport them to the shoot and not retain them in the belowground plant parts. Furthermore, they should tolerate high contents without showing symptoms of toxicity [99–104]. Cd, Co and Zn are efficiently transported from the roots to the shoot of the hyperaccumulator Solanum nigrum and are further redistributed within the shoot via the phloem [103]. Rapid uptake and release into the xylem are key properties of this species, while the redistribution via the phloem would be less important when whole shoots would be collected for phytoremediation [103].
Ligands were found to be important in hyperaccumulators for vacuolar sequestration and for the transport of heavy metals in the xylem [59,62,65]. Genetic engineering of the metabolism may lead to increased concentrations of ligands important for xylem loading and to decreased levels of ligands leading to vacuolar sequestration causing a retention of heavy metals in the roots [59,62,65,105,106]. Transporters for heavy metals or heavy metal complexes represent other targets for classical breeding and genetic engineering to improve properties of plants envisaged for phytoremediation [107,108].

7. Conclusions

Some translocators for heavy metals or heavy metal complexes are known, but we are far away from knowing the network of translocator proteins involved in the uptake into the roots, the subcellular distribution in roots and shoots, the release from the root symplast to the xylem, the uptake from the apoplastic into leaf cells and the loading into the phloem for further redistribution [78,109,110]. A more complete picture of transporters for heavy metals is highly desirable and may serve as a basis for genotype selection and breeding. Genetic tools available nowadays will presumably allow a rapid progress in this field.

The chemical forms (e.g., chelating agents) in various subcellular compartments as well as during long-distance transport in xylem and phloem should be known in more detail. The availability of complexing agents in a plant cell may be relevant for the retention in the cell (e.g., transport across the tonoplast and storage in the vacuole), the transfer to neighbor cells through plasmodesmata and the release into the apoplast for xylem or phloem loading. Sensitive localization techniques for heavy metals and metabolic studies for ligand availability may serve as a basis for more detailed studies [59,62–66,71,78,111].

Often interactions between various biotic and/or abiotic stresses affect plant growth and the composition of the biomass [112,113]. High salt concentrations or climatic factors such as drought or heat should be considered in more detail in the context of global change.

It will be a challenge for agronomists and for plant breeders to select appropriate genotypes for a given environment and to breed new genotypes with desired properties [114,115]. The harvested plant parts as well as the utilization of the plant biomass and not only heavy metal availability in the soil or other environmental factors are relevant in this context. Biofortification depends on the accumulation of desired micronutrients and on avoiding high levels of undesired heavy metals in plant parts harvested for human or animal nutrition [88,91]. Selective redistribution and accumulation processes for various heavy metals are the basis for well-balanced contents and must be considered for agronomic strategies, including plant breeding, genotype selection and agronomic practices [88,91]. Criteria for seed crops, grassland plants and heavy metal hyperaccumulators differ considerably. This must be adequately considered in basic research as well as in agronomic applications and breeding programs.

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Author Contributions

Valérie Page analyzed and reviewed heavy metal transport processes in cereals and legumes and contributed to writing the manuscript. Urs Feller analyzed and reviewed xylem–phloem interactions and heavy metal transport in hyperaccumulators and contributed to writing the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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