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Partitioning of zinc, cadmium, manganese and cobalt in wheat (*Triticum aestivum*) and lupin (*Lupinus albus*) and further release into the soil

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Abstract

The uptake and redistribution of the heavy metals zinc, cadmium, manganese and cobalt are relevant for plant nutrition as well as for the quality of harvested plant products. In the experiments reported here, seedlings of wheat (*Triticum aestivum* L.) and white lupin (*Lupinus albus* L.) were radiolabelled for 24 h with ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co via one seminal root (wheat) or via the main root (lupin). Plants were afterwards grown on rhizoboxes containing soil. Samples were collected throughout the experiment and analysed afterwards for their radionuclide contents. A strong retention in the labelled part of the root was observed for ^{57}Co in wheat and lupin and for ^{109}Cd in lupin, while ^{65}Zn and ^{54}Mn were transported to the shoot in both plants. While ^{65}Zn was redistributed via the phloem from older to younger leaves, ^{54}Mn accumulated in the first leaves and no major redistribution within the shoot was observed. ^{109}Cd was present in the shoot of wheat but not in the shoot of lupin. The redistribution of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in the phloem differed between wheat and lupin. The ^{65}Zn content in the wheat roots appearing after the labelling phase represented 34% of the total content in the plant at the end of the experiment and less than 3% remained in the labelled root, while a high percentage of ^{65}Zn was retained in the originally labelled part of the main root of lupin. Smaller quantities of ^{109}Cd, ^{54}Mn and ^{57}Co accumulated in all parts of the root system of wheat and lupin. Nevertheless, heavy metals were found in rhizosphere soil (1–2 mm soil around the roots) and bulk soil (no contact with roots) from both plants. Higher quantities of heavy metals were found in the rhizosphere soil close to the labelled part of the root. ^{65}Zn was present in large quantities in the rhizosphere soil close to all parts of the root system of wheat. For both plants, ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co were found in the bulk soil indicating that the plant itself might play a role in the redistribution of heavy metals in the soil around its own roots. Phloem-mobile elements may be transported to growing parts of the root system and may reach deeper soil layers. The redistribution of heavy metals in the soil may be in vertical and horizontal directions, at least as far as the root system grows.

Keywords: Bulk soil; Heavy metals; *Lupinus albus* L.; Rhizobox; Rhizosphere soil; Transport; *Triticum aestivum* L.

1. Introduction

Heavy metals (a group of metals with density higher than 5.0 g cm^{-3}; Sanità di Toppi and Gabbrielli, 1999) are components of the biosphere, and thus occur naturally in soils and plants. In most terrestrial ecosystems, both the underlying rock material and the atmosphere are two main natural sources of heavy metals (Schützendübel and Polle, 2002). While volcanoes and continental dusts are at the origin of heavy metals in the atmosphere (Schützendübel and Polle, 2002), heavy metals in soils are mainly due to human activities such as agricultural use of fertilizers, agrochemical compounds or sewage sludges, as well as activities like mining, combustion of fossil fuels and metals-working industries (Moreno-Caselles et al., 1997a; Schützendübel and Polle, 2002). Udom et al. (2004) reported that heavy metals such as zinc (Zn), lead (Pb), cadmium (Cd) and copper (Cu) added

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to soils through spreading of sewage sludge accumulate in soil layers from the surface to a depth of more than 65 cm. The fate of heavy metals in the soil is of great importance for the cycling of elements and for environmental quality.

Plants have a natural ability to extract ions from soil and to distribute them between the roots and the shoot. Within a certain concentration range, some heavy metals are essential for the growth of higher plants (Breckle, 1991). In this context, long-distance root-to-shoot transport in the transpiration stream via the xylem as well as the transfer from the xylem to the phloem and the retranslocation via the phloem must be considered as important processes for the redistribution of an element within a plant (Marschner, 1995). Recent studies have demonstrated that zinc is easily transported in the phloem of wheat (Herren and Feller, 1994, 1996; Pearson et al., 1995; Haslett et al., 2001) and that its redistribution may depend on the plant age and on the zinc content of the source organs (Herren and Feller, 1996). The redistribution of the manganese via the phloem may depend on the plant species and on its developmental stage (Herren and Feller, 1994). The mobility of cobalt in the phloem is considered to be intermediate (Marschner, 1995) and its distribution in the plant depends on the plant species (Moreno-Caselles et al., 1997a,b). Cadmium, which is very similar to zinc (Chesworth, 1991), may be taken up and transported in plants via similar pathways as zinc (Grant et al., 1998). However, most of the plants retain more than 50% of the absorbed cadmium in the roots (Obata and Umebayashi, 1993) and the long-distance translocation of cadmium may depend on the availability of other elements (Herren and Feller, 1997).

If it is known that heavy metals may be absorbed by the root system of the plant and that some of them may be transported to the shoot, the role of the plants in the redistribution of the heavy metals in the environment is a question that needs to be answered. The aerial part of the plant becoming senescent usually falls onto the soil surface. Due to the organic matter decomposition, it can be supposed that these plant parts may release substances as heavy metals, which may then accumulate at the soil surface. It can also be supposed that roots (alive and/or being decomposed) may release substances (heavy metals) into the soil. The only quantification of heavy metals in plants and soils will not allow a proper evaluation of their transport inside the plant and/or their release into the soil. For these reasons, we decided to use labelled heavy metals to follow precisely these transfers over a long period.

Two crops widely grown in agriculture, wheat (*Triticum aestivum* L. cv. Arina) and white lupin (*Lupinus albus* L. cv. Amiga, Südwestdeutsche Saatzucht, Rastatt, Germany), were chosen for this study. They differ in root morphological and physiological traits (Lersten, 1987; Clements et al., 1993) as well as in the response to adapt to various adverse environmental conditions by mycorrhizal colonization or by the formation of cluster roots (also called proteoid roots), for wheat and lupin, respectively (Alexander et al., 1988; Neumann et al., 1999, 2000; Lamont, 2003).

The two main aims of this study were (i) to characterize the transport of heavy metals in the different parts of the two plants and (ii) to evaluate the potential role of these plants in the redistribution of heavy metals in the environment. To this end, we were interested (a) to identify the transport of the zinc (Zn), cadmium (Cd), manganese (Mn) and cobalt (Co) from the roots to the shoot and their further redistribution within the shoot of wheat and lupin, (b) to compare the redistribution of these four heavy metals in the different parts of the root system of these two plants, (c) to determine if there is a release from the root system into the soil and (d) if the release of heavy metals is confirmed, to determine the spatial (horizontal and vertical) redistribution of the heavy metals in the plant/soil system. We investigated a pollutant heavy metal (Cd), which is found in many contaminated soils (Sauvé et al., 2000; Lugon-Moulin et al., 2004), two essential heavy metals (Zn and Mn) and the conditionally (for N_2 fixation) required Co.

2. Materials and methods

2.1. Germination and pre-growth

Dry grains of wheat (*T. aestivum* L. cv. Arina) were germinated on wet paper in darkness for two and a half days. Dry grains of white lupin (*L. albus* L. cv. Amiga, Südwestdeutsche Saatzucht, Rastatt, Germany) were incubated overnight in aerated de-ionized water before being placed in between filter paper sheets moistened with 0.2 mM $CaSO_4$ for 4 days in the dark and 1 day in a light (14 h, 190–250 $\mu E\,m^{-2}\,s^{-1}$)/dark (10 h) cycle.

2.2. Labelling and staining

For wheat, the labelling procedure used in our experiment has been described by Minder and Feller (2003). After germination, three seminal roots and the shoot of wheat plants had grown. Only the central seminal root was labelled with radionuclides, while the two other seminal roots were cut (this procedure caused no major effects on the further development of the plant and ensured that the radionuclides were taken up by only one root). The remaining root of the young plants (which was named main root at this time and throughout the text) was placed on nutrient solution containing radioactive heavy metals. The plants were labelled with a mixture of ^{65}Zn and ^{109}Cd (10571.42 and 10571.42 kBq/L, respectively) or a mixture of ^{54}Mn and ^{57}Co (2642.85 and 10571.42 kBq/L, respectively). After labelling for 24 h, the roots were washed (dipped three times sequentially in 100 mL of nutrient solution) to remove radioactive solutes from the root surface and then placed on a nutrient solution coloured with 0.1% Congo red to identify the root part initially labelled after further elongation (the Congo red had no effect on the plant growth). The seedlings were washed again and incubated 1 h in darkness on nutrient solution to allow the diffusion of the excessive

dye from the root apoplast. The young plants were then incubated on nutrient solution for 24 h to achieve a suitable size before the transfer into rhizoboxes.

The lupin roots were placed on nutrient solution containing radioactive heavy metals for 24 h in a light (14 h, 190–250 $\mu E\,m^{-2}\,s^{-1}$)/dark (10 h) cycle. The lupin plants were labelled with a mixture of ^{65}Zn and ^{109}Cd (462.5 and 462.5 kBq/L, respectively) or a mixture of ^{54}Mn and ^{57}Co (102.8 and 411.1 kBq/L, respectively). The volume of the nutrient solution with radionuclides was higher than for wheat due to the size of the root of lupin ranging from 8 to 10 cm and only 1–2 cm for wheat. After labelling, the roots were washed to remove radioactive solutes from the root surface, incubated for 2 h in a nutrient solution containing 0.1% Congo red and re-washed to allow the diffusion of the excessive dye from the root apoplast before transferring into rhizoboxes.

2.3. Nutrient supply

The nutrient solution used for each step of labelling and staining contained 0.5 mM $Ca(NO_3)_2$, 0.188 mM K_2SO_4, 0.332 mM $MgSO_4$, 10 μM FeNaEDTA, 12.5 μM KCl, 7.4 μM H_3BO_3, 2.8 μM $MnSO_4$, 0.4 μM $CuSO_4$, 0.45 μM $ZnSO_4$ and 0.08 μM $Mo_7O_{24}(NH_4)_6$. This nutrient solution was also used for watering the rhizoboxes over the incubation time and the lack of phosphorus was applied to enhance the growth of proteoid roots in white lupin plants.

2.4. Rhizobox experiment

The soil was collected from an agroecosystem (Corcelles-Concise, VD, Switzerland) from 5 to 30 cm depth (Ap horizon) of the soil profile. This loamy soil (50% sand, 30% silt and 20% clay), is a chromic luvisol (FAO) with the following characteristics: organic carbon: 0.97%, mineral carbon: 0.31%, C/N: 11.8 and pH_{H_2O}: 7.8.

The soil was air-dried and sieved at 2 mm before filling the rhizoboxes which consisted of two transparent Plexiglas plates (20 cm width and 30 cm height) separated by Plexiglas spacers of 1 cm (Fig. 1) and the bottom was closed with a nylon mesh to avoid soil losses (Weisskopf et al., 2005). Each rhizobox was filled with dried soil (approximately 630 g) up to 1 cm below the top. One day before the transfer of the plants, rhizoboxes were watered with de-ionized water. Seedlings of wheat and lupin were placed in separate rhizoboxes between the front Plexiglas plate and a 25 μm-nylon mesh (SEFAR NITEX 03-25/19), one plant per rhizobox. This procedure allowed the separation of the whole root system from the soil, but the roots were still in contact with the soil via the root hairs, which can be very long (from 80 to 1500 μm) with a diameter from 5 to 17 μm (Dittmer, 1949) and are able to pass through the nylon mesh. All rhizoboxes were set up vertically in darkened plastic containers and protected against light, and the experiment was conducted with a photoperiod of 14 h light (190–250 $\mu E\,m^{-2}\,s^{-1}$) and 10 h darkness. The room temperature was 24 °C during the day and 23 °C during the night.

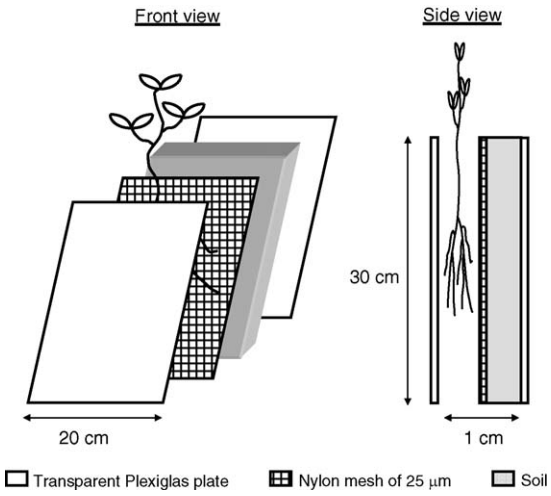

Fig. 1. Scheme of a rhizobox. In the side view, the space between the front plexiglas plate and the nylon mesh (1 mm) is enlarged.

Over the incubation time, each rhizobox was watered with 100 mL of the nutrient solution two times per week, and a sample of 20 mL of the exceeding solution was collected.

2.5. Plant and soil analyses

Plant and soil samples were collected 4, 8, 12, 20, 28 and 50 days after labelling with radioactive heavy metals. The experiment with the lupin plants ended at day 28 when the biomass reached a critical level. Indeed, on lupin plants older than 28 days, cotyledons and first leaves were often missing as a consequence of senescence and abscission. Three plants of both wheat and lupin were harvested immediately after the labelling process (day 0). At each date, three rhizoboxes were randomly chosen and opened to collect the plants and the soil sections. The wheat plants were dissected into several pieces: (i) the labelled part of the main root (labelled with radionuclide and stained in red), (ii) the apical part of the main root including its lateral roots (unstained), (iii) the lateral roots outgrowing from the labelled part of the main root, (iv) the other roots (other seminal and adventitious roots), (v) the grain, (vi) the scutellum (including scutellum, small shoot axis and shoot apex), (vii) the coleoptile and (viii) leaf 1 (oldest leaf) to leaf 10 (youngest leaf, expending at the end of the experiment). No tillers were produced during the experiment. Oldest leaves (leaves 1, 2, 3 and sometimes leaf 4) were senescent at the end of the experiment (day 50). The lupin plants were dissected into: (i) the labelled part of the main root (labelled with radionuclides and stained in red), (ii) the apical part of the main root including its lateral roots, (iii) the lateral roots outgrowing from the labelled part of the main root, (iv) the cluster roots, (v) the hypocotyl, (vi) the cotyledons, (vii) the stem and (viii) leaf 1 (oldest leaf) to leaf 15 (youngest leaf, expanding at the end of the experiment). Oldest leaves (leaves 1 and 2) were sometimes senescent at

the end of the experiment (day 28). All these samples were dried at room temperature and the radioactivity was analysed after completing the experiment.

Facing plant roots of wheat and lupin, a thin soil layer was stuck onto the nylon mesh. In addition, the roots themselves let some traces on the surface of the soil. The soil adhering to the nylon and soil linings created by roots were considered as the rhizosphere soil, i.e. the absorbing root–soil interface close to the root system.

From the wheat experiment, four sections of rhizosphere soil were collected and codified as following: (i) rhizosphere soil from the labelled part of the main root: RSLP, (ii) rhizosphere soil from the lateral roots outgrowing from the labelled part of the main root: RSLR, (iii) rhizosphere soil from the apical part of the main root: RSAP and (iv) rhizosphere soil from the other roots: RSOR. From the lupin experiment, four sections of rhizosphere soil were collected and codified as following: (i) rhizosphere soil from the labelled part of the main root: RSLP, (ii) rhizosphere soil from the lateral roots outgrowing from the labelled part of the main root: RSLR, (iii) rhizosphere soil from the apical part of the main root: RSAP and (iv) rhizosphere soil from the cluster roots: RSCR. The radioactivity contained in the rhizosphere soil was analysed also after completing the experiment.

For the soil farer from the root system, i.e. the bulk soil, three soil sections of around 210 g (dry matter) were defined with respect to the distance from the top to the bottom of the rhizobox and codified as following: (i) the first section, from the top of the rhizobox to 10 cm depth: bulk soil 0–10, (ii) from 10 to 20 cm: bulk soil 10–20 and (iii) from 20 to 30 cm: bulk soil 20–30. The soil sampled in each section was homogenized and dried at a temperature lower than 35 °C and 10 g were then used for gamma counting.

The samples of plant parts, of rhizosphere soil and of bulk soil were analysed at the same time at the end of the experiments. The radioactivity contained in the samples was counted with a gamma counter (1480 Wizard 3″, Wallac Oy, Turku, Finland), an automatic counter containing a strong lead shielding around the detector. The remaining background was measured and subtracted from the sample value. The measurements were corrected for the decay to a reference date to avoid artefacts caused by the different half-lifes of the radionuclides ($t_{1/2}$ (^{65}Zn) = 244.26 days; $t_{1/2}$ (^{109}Cd) = 462.6 days; $t_{1/2}$ (^{54}Mn) = 312.3 days; $t_{1/2}$ (^{57}Co) = 271.79 days).

2.6. Statistical analysis

Values of heavy metal contents are means of three replicates. The relative radionuclide contents were tested for the wheat and lupin separately. Analysis of variance was performed for the roots, the shoot, the total plant, the rhizosphere soil, the bulk soil and the total soil and least significant differences were calculated (Statistix for Windows, Version 1.0, Analytical Software, Tallahassee FL; rejection level 0.05, $n = 3$).

3. Results

3.1. Dynamics of heavy metals in whole plants of wheat and lupin

The four radionuclides, ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co supplied to the young wheat plants were re-allocated differently (Fig. 2). The quantity of ^{65}Zn decreased quickly in the labelled part of the main root that was the counterpart of an

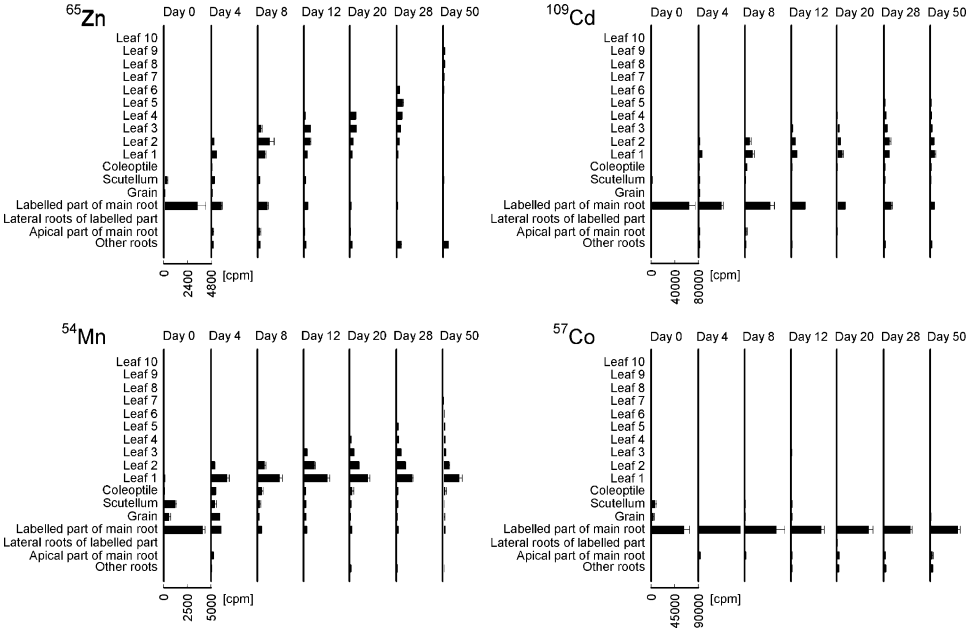

Fig. 2. Dynamics of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in young wheat plants. Means and standard errors of three replicates are shown for the radionuclide contents (cpm, counts per minute) in the different plant parts.

increase in its apical part, the other roots and in the shoot. The ^{65}Zn content in the roots appearing after the labelling phase (mentioned "other roots" in the figure) represented 34% of the total content of the plant at the end of the experiment (day 50). A large amount of ^{65}Zn moved towards the leaves during their expansion. However, with leaf aging, the content of ^{65}Zn decreased in the wheat leaves. The ^{109}Cd content in the labelled part of the main root decreased more slowly over the time than that for ^{65}Zn and 20% of the total ^{109}Cd content in the plant remained in the labelled part at day 50. Only a minor part of ^{109}Cd moved towards the apical part of the main root and/or the other roots, while it increased considerably and remained afterwards constant in the older leaves. ^{54}Mn decreased more quickly in the labelled part of the main root than ^{65}Zn and ^{109}Cd and increased simultaneously in leaf 1. The amount of ^{54}Mn observed in the scutellum and in the grain at day 0 decreased during further growth. At the opposite, ^{54}Mn in leaf 1 remained at a constant level throughout the experiment, while small quantities accumulated in the other leaves during the incubation time. Finally, 80% of the total ^{57}Co present in the plant were retained in the labelled part of the main root till day 50 whereas the apical part of the main root and the other roots contained around 14% of ^{57}Co.

The dynamics of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in lupin differed from those in wheat (Figs. 2 and 3). The amount of ^{65}Zn in the labelled part on the main root decreased slowly to about 50% of the initial content at day 28 (Fig. 3). Small quantities were found in the lateral roots of the labelled part, in the apical part of the main root and in the cluster roots. As for the wheat, ^{65}Zn moved towards the leaves during their expansion and, in a later phase, moved to the youngest leaves. With regards to the ^{109}Cd redistribution, it was slower in lupin than in wheat. About 90% of the total ^{109}Cd present in the plant remained in the labelled part of the main root till the end of the experiment and the last 10% were located in the lateral roots of the labelled part. The content of ^{54}Mn decreased considerably in the labelled part of the main root, a large amount being transported to the oldest leaves (leaves 1–3) and remained there afterwards. A little amount of ^{54}Mn moved into the leaves 4–6. The ^{54}Mn present in the hypocotyl at the beginning of the experiment (day 0) decreased thereafter. Concerning ^{57}Co, the content in the labelled part of the main root decreased slowly and represented 68% of the total content in the plant at day 28. Only small quantities of this heavy metal reached the hypocotyl, the leaves one to nine or the lateral roots of the labelled part.

3.2. Release of heavy metals in the soil sections

From previous experiments with hydroponic cultures of lupin and wheat (Page and Feller, 2005), it appeared obvious that these plants exude heavy metals from their roots. To identify from which part of the root system these radionuclides were excreted, different parts of the rhizosphere soil and of the bulk soil were analysed (Figs. 4 and 5).

In wheat, ^{65}Zn amounts in the RSLP, in the RSAP and in the RSOR were higher than the amount in the RSLR throughout the experiment (Fig. 4). At day 50, the content of ^{65}Zn in RSOR exceeded those in the RSLP. Regarding ^{109}Cd, its content in the RSLP was the highest compared to rhizosphere soils from the other parts of the root system. The release of ^{54}Mn and ^{57}Co to the rhizosphere soil was similar to that of ^{109}Cd except for the RSOR, where ^{54}Mn and ^{57}Co (but not ^{109}Cd) where found in considerable quantities.

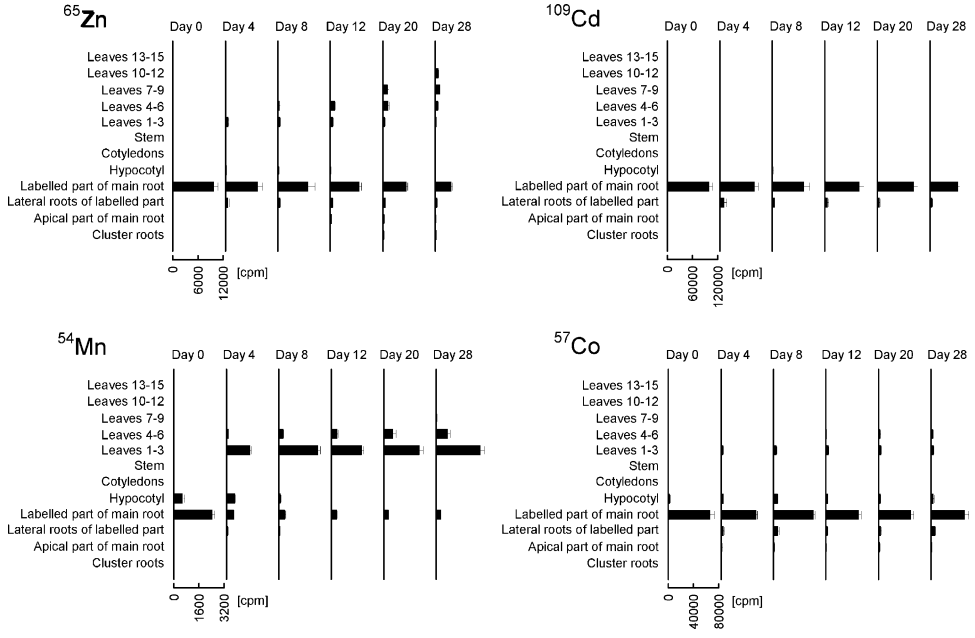

Fig. 3. Dynamics of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in young plants of white lupin. Means and standard errors of three replicates are shown for the radionuclide contents in counts per minute (cpm).

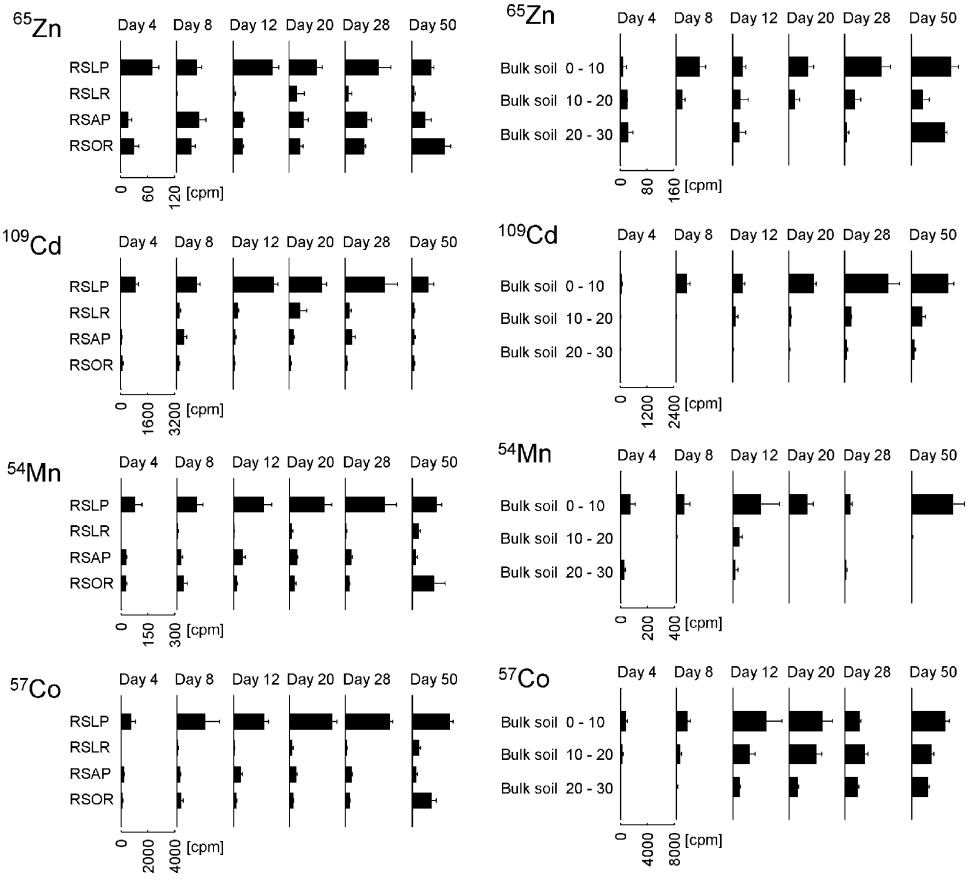

Fig. 4. Content of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in the rhizosphere and bulk soil of wheat plants. The radionuclide contents in the rhizosphere soil are shown at the left side and are codified as following: rhizosphere soil from the labelled part of the main root: RSLP, rhizosphere soil from the lateral roots outgrowing from the labelled part of the main root: RSLR, rhizosphere soil from the apical part of the main root: RSAP and rhizosphere soil from the other roots: RSOR. The radionuclide contents in the soil far from the roots are shown at the right side: first section from the top of the rhizobox and 10 cm depth (bulk soil 0–10), second section from 10 to 20 cm (bulk soil 10–20) and third section from 20 to 30 cm (bulk soil 20–30). Means and standard errors of three replicates are shown for the radionuclide contents (cpm, counts per minute) in the different soil sections.

In the case of the soil far from the root system of the wheat, the bulk soil 0–10 contained the largest quantity of all radionuclides. Large amounts of ^{65}Zn and ^{57}Co were also found in the two other parts of the bulk soil, especially at day 50 where the amount of ^{65}Zn in the bulk soil 20–30 was close to that in the bulk soil 0–10. Small quantities of ^{109}Cd and ^{54}Mn were detected in the bulk soil 10–20 and in the bulk soil 20–30 in comparison to ^{65}Zn and ^{57}Co.

In the rhizosphere soil near the lupin roots (Fig. 5), all the four radionuclides were found in large quantities in the RSLP, while the contents of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in the rhizosphere soil from other parts of the root system remained very low. Although the various heavy metals were distributed similarly in the rhizosphere soil, the relative distribution in the three bulk soil layers differed considerably. The heavy metals ^{65}Zn and ^{109}Cd were largely present in bulk soil 0–10, but much less elsewhere. In contrast to ^{65}Zn and ^{109}Cd, ^{54}Mn and ^{57}Co were also detected in rather large quantities in the two other soil sections. At day 28, more ^{54}Mn was quantified in the soil layer close to the bottom of the rhizobox (bulk soil 20–30) than in the top layer (bulk soil 0–10).

3.3. Overview of heavy metal contents in plant parts and soil sections

In order to better understand the redistribution of the heavy metals, and to draw up a balance sheet of our experiment, Table 1 summarizes the relative contents of the four heavy metals in the plant fragments (roots and shoot) and in the various soil fractions (rhizosphere and bulk soils). A general observation for wheat and lupin was that more than 70% of the heavy metals contained in the plant–soil system remained in the whole plant, while percentages ranging from 3.3% (for ^{109}Cd in lupin) to 26.2% (for ^{54}Mn in lupin) of these heavy metals were released into the soil. In both plants, ^{54}Mn was the radionuclide more rapidly released from the labelled part of the root. Indeed, 67.2 and 80.3% of this metal in lupin and wheat, respectively, moved from the root to the shoot. In lupin, ^{54}Mn was in higher quantities released from the root system into the soil (26.2%). In the case of ^{57}Co, a strong retention in the root was observed (77.5% in wheat and 71.7% in lupin) while 18.9% (wheat) and 10.0% (lupin) were released from the roots into the soil. In contrast to ^{54}Mn

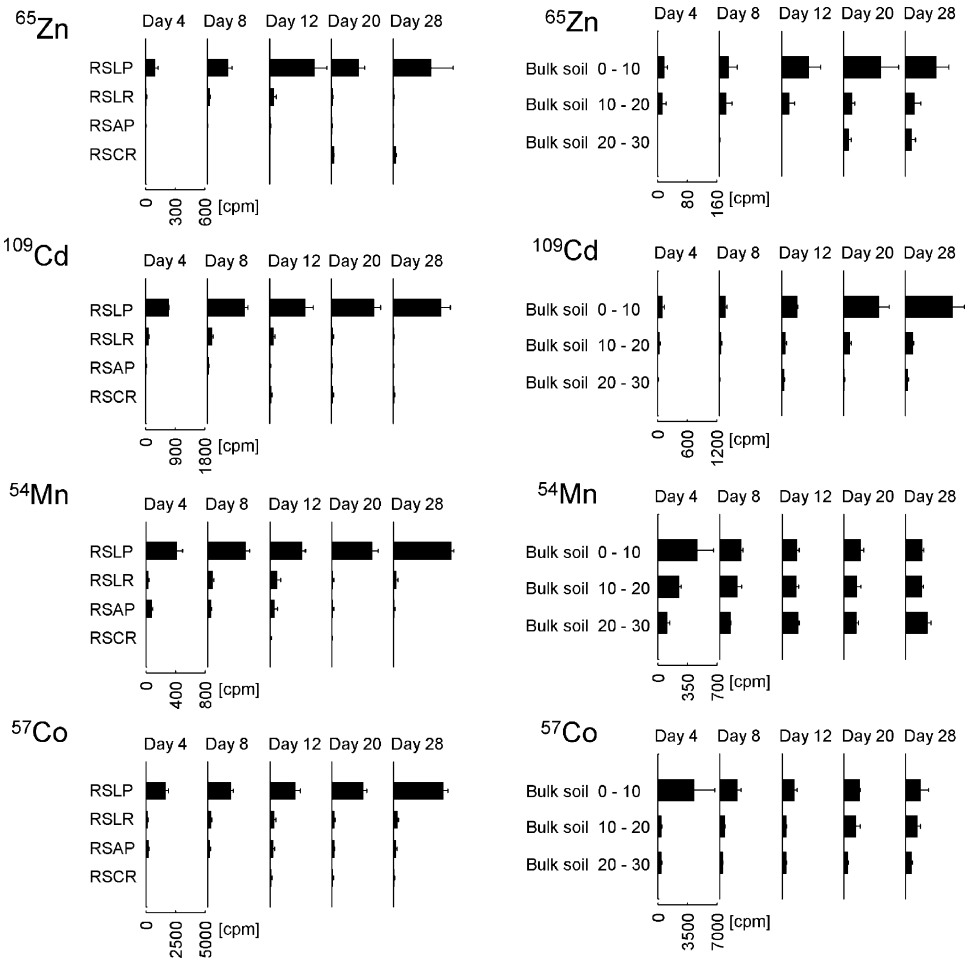

Fig. 5. Content of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in the rhizosphere and bulk soil of the white lupin plants. The radionuclide contents in the rhizosphere soil are shown at the left side and are codified as following: rhizosphere soil from the labelled part of the main root: RSLP, rhizosphere soil from the lateral roots outgrowing from the labelled part of the main root: RSLR, rhizosphere soil from the apical part of the main root: RSAP, rhizosphere soil from the cluster roots: RSCR. The radionuclide contents in the soil far from the roots are shown at the right side and are codified as following: first section from the top of the rhizobox and 10 cm depth (bulk soil 0–10), second section from 10 to 20 cm (bulk soil 10–20) and third section from 20 to 30 cm (bulk soil 20–30). Means and standard errors of three replicates are shown for the radionuclide contents (cpm, counts per minute) in the different soil sections.

Table 1
Relative contents (% of total radionuclide per rhizobox) of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in the plant and in the soil fractions at the last day of the experiments (day 50 for wheat and day 28 for lupin)

Plant day	Radionuclide	Relative contents (% of total content per rhizobox)					
		Plant			Soil		
		Roots	Shoot	Total	Rhizosphere soil	Bulk soil	Total
Wheat day 50	^{65}Zn	30.1 ± 1.5 b	49.7 ± 1.8 c	79.8 ± 1.9 b	7.7 ± 0.6 a	12.5 ± 1.6 a	20.2 ± 1.9 a
	^{109}Cd	28.4 ± 2.7 b	61.7 ± 4.2 b	90.1 ± 1.5 a	3.9 ± 1.2 b	6.0 ± 1.2 b	9.9 ± 1.5 b
	^{54}Mn	9.0 ± 0.7 c	80.3 ± 0.5 a	89.3 ± 1.1 a	6.3 ± 0.7 ab	4.5 ± 0.9 b	10.7 ± 1.1 b
	^{57}Co	77.5 ± 1.1 a	3.6 ± 1.4 d	81.1 ± 1.1 b	6.2 ± 0.4 ab	12.7 ± 0.6 a	18.9 ± 1.1 a
Lupin day 28	^{65}Zn	56.8 ± 2.0 c	37.2 ± 2.6 b	94.0 ± 2.3 ab	4.6 ± 1.9 bc	1.4 ± 0.4 c	6.0 ± 2.3 bc
	^{109}Cd	93.4 ± 0.5 a	3.4 ± 0.2 d	96.7 ± 0.4 a	1.8 ± 0.2 c	1.4 ± 0.2 c	3.3 ± 0.4 c
	^{54}Mn	6.6 ± 0.4 d	67.2 ± 1.4 a	73.8 ± 1.3 c	14.7 ± 0.6 a	11.5 ± 1.0 a	26.2 ± 1.3 a
	^{57}Co	71.7 ± 2.3 b	18.3 ± 1.8 c	90.0 ± 2.0 b	5.5 ± 0.9 b	4.5 ± 1.1 b	10.0 ± 2.0 b

Means and standard errors of three replicates are shown. Values in the same column (for wheat and for lupin separately) followed by the same letter are not significantly different at the $P = 0.05$ level.

and ^{57}Co, the behaviour of ^{65}Zn and ^{109}Cd in the plant parts and their release into the soil differed in both plants. ^{65}Zn is more mobile in the wheat than in the lupin. Indeed 49.7% of ^{65}Zn were transferred to the shoot and 20.2% were released into the soil. ^{109}Cd was strongly retained in the roots of lupin (93.4%) while only 28.4% of this radionuclide stayed in the roots of wheat.

4. Discussion

In this study, the dynamics of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in wheat and lupin plants were characterized. These four heavy metals behaved differently with regard to their root-to-shoot transfer, their redistribution in the shoot and in the root system and their release into the soil.

4.1. Heavy metal root-to-shoot transport

A large amount of the heavy metals (except ^{109}Cd in lupin and ^{57}Co in wheat and lupin) moved from the labelled root into the leaves indicating that these radionuclides were transported in the transpiration stream via the xylem. The velocity of this transport was not identical for all heavy metals. For example, the root-to-shoot transfer of ^{54}Mn was rapid in wheat and lupin, while in the case of ^{65}Zn, this transfer was considerably slower in lupin than in wheat. It might be suggested that the requirement of an element in the rapidly growing tissues of the plant may explain the different velocity of the heavy metal transfer from the roots to the shoot.

The strong retention of ^{109}Cd observed in white lupin roots was in agreement with previous results (Römer et al., 2000, 2002; Ximénez-Embún et al., 2002; Zornoza et al., 2002). Surprisingly, more than 60% of ^{109}Cd moved from the roots to the shoot in wheat (Table 1). It may be suggested that wheat and lupin have different translocation processes such as those including Cd transport out of xylem parenchyma cells into the xylem system, or Cd loading into and out of the phloem cells. Another explanation is that cadmium may be recognized as a toxic compound by the roots of both wheat and lupin, thus leading to the activation of mechanisms such as sequestration in the vacuole or in the cell walls (Sanità di Toppi and Gabbrielli, 1999), especially in lupin, in order to avoid an accumulation of cadmium in the shoot. Cadmium is indeed known to have several toxic effects on plants (Sanità di Toppi and Gabbrielli, 1999, and references therein).

The negatively charged cell walls can attract mobile cations (Wang and Evangelou, 1995). The affinity between cations and plant cell walls do not necessarily occur in the same order for all plant species. Indeed, interactions between cations and cell walls may vary considerably depending on the plant species or genotype (Wang and Evangelou, 1995). This fact may explain the different retention of ^{109}Cd and ^{57}Co in the roots of wheat and lupin.

4.2. Redistribution of heavy metals within the shoot

Although ^{65}Zn and ^{54}Mn (in wheat and lupin) and ^{109}Cd (only in wheat) were released from the roots to the shoot, the ^{65}Zn alone was redistributed from oldest leaves to youngest leaves, mainly via the phloem. These results are in line with previous studies showing that Zn is well mobile in the phloem (Herren and Feller, 1994; Haslett et al., 2001; Riesen and Feller, 2005). Other authors demonstrated that Cd and Mn are also mobile in the phloem (Cakmak et al., 2000; Reid et al., 2003; Riesen and Feller, 2005). In the study presented here, the low ^{109}Cd content in the youngest wheat leaves (Fig. 2) might derive from the slow release from the root system. In this case, an export out of the old leaves via the phloem might be compensated by an import into these old leaves directly from the roots via the xylem, resulting in a masking of the movement of this radionuclide via the phloem from old leaves to young leaves. Such a movement was clearly demonstrated for ^{65}Zn by the decrease of the content in the oldest leaves.

4.3. Redistribution of heavy metals within the root system

The redistribution of heavy metals in the different parts of the root system is of great importance in the context of the release of heavy metals into the soil. The presence of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co in roots appearing after the labelling phase lead to the conclusion that these heavy metals were translocated via the phloem from the labelled part of the main root into the other parts of the root system. Some previous studies support this hypothesis (Welch et al., 1999; Haslett et al., 2001). These results added to ours lead to the conclusion that the loading of heavy metals into the phloem must be considered as a key process involved in the redistribution within the root system.

4.4. Release of heavy metals into the soil and distribution within the soil

A part of the heavy metals ranging from 3.3% (^{109}Cd in lupin) to 26.2% (^{54}Mn in lupin) was released from both wheat and lupin plants into rhizosphere and bulk soil (Figs. 4 and 5). Several hypotheses may explain this release of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co into the soil: (a) ions bound to cell wall components may be re-solubilized (relevant for initially labelled part of the root system), (b) cells themselves may release radionuclides into the soil across the intact plasma membrane and (c) radionuclides may come from senescing cells following the membrane disruption. In our study, wheat plants released more heavy metals into the soil than lupin, except in the case of ^{54}Mn. The general morphology of the root system of the two plants differs. Wheat has a diffuse root system consisting in adventitious roots (including the so-called seminal embryo roots) that branch and rebranch to form several orders of slender roots (Lersten, 1987). Some roots extend horizon-

tally for 30–35 cm just below the surface while other roots grow diagonally downward within the top 30 cm soil. Below 30 cm, fewer and less branched roots extend vertically to 150 cm or deeper (Lersten, 1987). Regarding white lupin, this plant has a strong taproot which can penetrate soil to depths greater than 1 m and an extensive first-order lateral root system also penetrating deeply into the soil profile. Cluster roots occur mainly on these first-order lateral roots and occasionally on the taproot (Clements et al., 1993). The morphological differences in the root systems of the two plants could explain the different releases. Indeed, the diffuse root system of wheat has a larger surface in contact with the soil than the taproot of lupin. Moreover, the thin roots of wheat may be more easily disrupted in the soil than the thick roots of lupin. Another difference is the formation of cluster roots by lupin. Interestingly, only traces of heavy metals were found in cluster roots and in the rhizosphere soil collected close to these special roots. Excretion of organic acids does not seem to play a role in the release of heavy metals from the cluster roots into the soil.

As mentioned in the literature, growing roots may release different compounds into the rhizosphere. For example, graminaceous species release phytosiderophores for the mobilization of Fe and other metals such as Zn, Ni and Cd (Marschner, 1995; Römheld and Awad, 2000). On the other hand, when exposed to a phosphorus deficiency, white lupin plants usually produce clusters roots, which exude large amounts of citric and malic acids (Neumann et al., 1999). Root exudation is considered to be responsible for the chemical mobilization of nutrients in the rhizosphere (Neumann et al., 1999). Considering these facts, the question arises whether these compounds may play a role in the transfer of ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co from the rhizosphere soil to the bulk soil, although normally these mechanisms are involved in an increased acquisition of nutrients.

For all of the radionuclides, the quantity present in the rhizosphere soil close to the labelled part of the main root was high (Figs. 4 and 5). These high amounts of heavy metals may be explained by a release from the labelled part of the main root (e.g. from the root apoplast or from senescing root hairs). Another possibility is that living root hairs may be separated from the root during the opening of the rhizobox for collecting plant and soil samples. The radioactivity contained in these root hairs may then contribute to the level in the rhizosphere soil. This latter possibility would be only relevant for a limited time in the development of a root part when root hairs are formed and active.

Only trace amounts of the four radionuclides were found in the solution passing through the rhizoboxes (data not shown). This minor mass-flow is not sufficient to explain the transfer of heavy metals to the bottom part of the rhizoboxes. Indeed, different quantities of ^{54}Mn and ^{57}Co found in the bulk soil 20–30 of the wheat experiment, as well as the different quantities of ^{65}Zn and ^{109}Cd found in the different rhizosphere soils (RSAP and RSOR) of the wheat experiment (in both cases the soils came from the same rhizoboxes), reinforce the hypothesis that the radionuclides were transported in the root system and then released into the soil. However, the high quantities of ^{65}Zn in the bottom part of the bulk soil (bulk soil 20–30) in the wheat experiment are in agreement with those found both in originally unlabelled roots and in rhizosphere soil close to these roots (Figs. 2 and 3). Indeed, most of these roots were present in the bottom part of the rhizobox at the end of the experiment. These results lead to the conclusion that a high phloem mobility of a radionuclide lead to a high content in newly formed parts of the root system and to a release from the roots into the soil.

In conclusion, it appears that ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co were redistributed in the shoot and in the root system of wheat and lupin plants. The release into the xylem and the loading into the phloem are important mechanisms in this context. Moreover, ^{65}Zn, ^{109}Cd, ^{54}Mn and ^{57}Co were released into the rhizosphere and bulk soils. The results presented here show that plants may play a role in the redistribution of heavy metals in the environment. Heavy metals present in the atmosphere may be deposited on the surface of the plants and enter the shoot. Afterwards, they may either remain in the leaves and be released on the surface of the soil when the leaves fall or may be transported from the shoot to the root system via the phloem as demonstrated by Riesen and Feller (2005). Then, heavy metals in the roots may be released from living roots into the soil or may remain in the soil after the death of the roots. Plants may also take up heavy metals from a contaminated region of the soil and transport these elements via the root system to a non-contaminated part of the soil. This redistribution of heavy metals in the soil depends on the spatial arrangement of the roots. In this context, the morphology of the root system is important for the volume of soil that may be contaminated by a release from the roots. Heavy metals present at the soil surface after the application of sewage sludge or fertilizers may be redistributed in the root system of plants growing on this soil and reach soil layers far below the surface. The next step would be to use rhizoboxes containing soil contaminated with heavy metals in order to be nearer to the natural conditions.

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