ROOT DISTRIBUTION AND UPTAKE OF SURFACE-APPLIED RADIONUCLIDES BY MAIZE IN FIELD SOILS

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### List of abbreviations and symbols

- **b**: soil buffer power, (dimensionless)
- **$C_h$**: ion initial concentration in soil solution, (mmol dm$^{-3}$)
- **$C_{min}$**: concentration of ion where net influx $I_n=0$, (µmol cm$^{-3}$)
- **$C_{si}$**: initial concentration of labile ions in the soil, (mmol dm$^{-3}$)
- **$D_e$**: effective diffusion coefficient, (cm$^2$ s$^{-1}$)
- **$D_L$**: diffusion coefficient in water, (cm$^2$ s$^{-1}$)
- **$f$**: impedance factor for ion diffusion through soil, resulting from tortuosity, water density, and surface charges, (dimensionless)
- **FES**: frayed edge sites
- **$I_n$**: net influx of ions into plant roots, (µmol cm$^{-2}$ s$^{-1}$)
- **$I_{max}$**: maximal net influx of ions into roots, (µmol cm$^{-2}$ s$^{-1}$)
- **$k$**: rate of root growth, (cm s$^{-1}$)
- **$K_m$**: Michaelis-Menten constant; ion concentration in solution $C-C_{min}$ where $I_n=1/2 I_{max}$, (µmol cm$^{-3}$)
- **$L_0$**: length of root per unit soil volume, (cm cm$^{-3}$)
- **LDS3**: leaf development stage 3
- **OC**: organic carbon
- **PFP**: preferential flow paths
- **PS**: pollen shed
- **$r$**: radial distance from root axis, (cm)
- **$r_0$**: root radius, (cm)
- **RES**: regular exchange sites
- **SA**: specific activity
- **$t$**: time, (day or seconds)
- **$V_0$**: water uptake velocity, (mL cm$^{-2}$ s$^{-1}$)
- **$\Theta$**: volumetric water content, (m$^3$ m$^{-3}$)
- **ZFP**: zone di flusso preferenziale
Abstract

Risk assessment models for the soil-to-plant transfer of radionuclides rely on the assumption that soils are homogenously contaminated. However, field soils are characterized by a high degree of heterogeneities mainly caused by macro-structure which can affect the distribution of surface-applied radionuclides and their accessibility by plant roots. The main objective of this thesis was to evaluate the influence of the heterogeneous distribution of surface-applied radionuclides and their spatial relation with roots on the soil-to-plant transfer of four radionuclides ($^{54}$Mn, $^{57}$Co, $^{65}$Zn and $^{134}$Cs). The analysis of the distribution of surface-applied radionuclides was carried out using dye tracers which allowed the visualization of the structure-induced water flow paths. $^{54}$Mn, $^{57}$Co, $^{65}$Zn, and $^{134}$Cs have been applied on the surface of two untilled agricultural soils located in Switzerland and maize (Zea mays L. cv. Corso) was used as model plant. Root distribution and the spatial interrelation with the preferential flow paths (PFP) have been carried out by in-situ mapping technique.

We questioned the relevance for real field conditions of the standard experimental approach advised by the International Atomic Energy Agency to obtain data for the transfer factor parameter which is used in the mathematical model to quantify the soil-to-plant transfer of radionuclides. Standard experiments carried out in greenhouses with plants grown in sieved and homogeneously contaminated soils. We analyzed the effect of soil macro-structure on the recovery of $^{54}$Mn, $^{57}$Co, $^{65}$Zn and $^{134}$Cs in the aerial part of maize grown in an untilled agricultural soil in comparison to the recovery in the aerial part of maize grown in the greenhouse and on the same soil that was sieved and homogeneously labeled before being repacked in pots. We observed a heterogeneous distribution of the surface-applied radionuclides due to the structure-induced heterogeneous water flow, and a moderate (10 to 15%) occurrence of roots in these areas of radionuclides enrichment. A significantly higher recovery of $^{57}$Co (2-fold) and $^{134}$Cs (10-fold) was observed in the plants grown in the field soil, whereas no differences in the recovery of $^{54}$Mn and $^{65}$Zn between the two experiments was detected.

Risk assessment models for the soil-to-plant transfer of radionuclides do not take into account the time-dependent variations of radionuclides accessibility by roots occurring at the soil-root interface. We studied the redistribution of $^{54}$Mn, $^{65}$Zn, $^{57}$Co, and $^{134}$Cs in the soil profile and their uptake by maize grown on an untilled agricultural soil during a growing season. Surface-applied radionuclides were concentrated in the preferential flow paths in comparison to the soil matrix due to structure-induced non-uniform water flow. However, the amount and the seasonal distribution of the precipitation promoting the convective vertical displacement of radionuclides and their infiltration into the soil matrix caused an expansion of the PFP and a decrease of radionuclides concentration in these areas with time. Only a small fraction (15-20%) of the root system was located within the preferential flow paths. Results of the study of the time-dependent variation of the recovery of the surface applied radionuclides during an entire maize growth cycle varied
depending on the radionuclide. The recovery of $^{54}$Mn in the aerial part of maize increased with time showing significantly higher values at each subsequent harvest. The recovery of $^{57}$Co and $^{65}$Zn in the aerial part of maize increased from the first (leaf development stage 3) to the second harvest (pollen shed), while it was constant between pollen shed and maturity. $^{134}$Cs was not detectable in the aerial part of maize at LDS3 and at maturity.

Results obtained in the field experiments have shown that only a small fraction (10 to 15%) of the root system was located in the preferential flow pathways where radionuclides were concentrated in respect to the soil matrix. This suggested that the roots located in these areas were responsible for the uptake of radionuclides. A split-root experiment was used to simulate the heterogeneous distribution of $^{134}$Cs and root. A single root was grown in a $^{134}$Cs contaminated compartment, while the rest of the root system was grown in an uncontaminated compartment. Plants with the whole root system growing in a solution contaminated with $^{134}$Cs were used as control. We tested the effect of the competition between Cs and K on the uptake and translocation of $^{134}$Cs by using two target potassium concentrations: one at 0.2mM and one at 1.05mM. The single root (equivalent to 21% of the dry matter of the control root) was able to take up 50% of the amount of $^{134}$Cs taken up by the control root; the quantity translocated to the shoots was equal to 47% of that of the control shoot. This effect was about 3 times weaker in the higher K treatment (1.05mM).

The Barber-Claassen model was used to predict the uptake of $^{134}$Cs and K by maize at leaf development stage 3 and at pollen shed. Predicted value of the recovery of $^{134}$Cs of plant at pollen shed was one order of magnitude higher than the measured value, while the predicted value of the recovery of K was in the same order of magnitude of that measured but two-times higher. K and $^{134}$Cs uptake by maize at leaf development stage 3 could not be predicted with the model because it was not possible to obtain the Michaelis-Menten parameters for plant at this development stage.

In view of these results, it appears that the soil-to-plant transfer of radionuclides in field soils can be explained by various factors; the effect of the soil macrostructure on the distribution of radionuclides and roots represents only part of the factors influencing it. Other processes are involved in the dynamics between the soil, the root, and the radionuclides, such as: (i) environmental conditions (i.e. amount and seasonal distribution of rainfall), (ii) the activity of rhizosphere microbes and mycorrhizae fungi, (iii) the recirculation of the absorbed ions within the plant’s organs, and (iv) competing ions.
Riassunto

I modelli matematici utilizzati per predire il rischio associato al trasporto di isotopi radioattivi dal suolo alla pianta considerano il suolo come un substrato omogeneo in cui gli isotopi radioattivi sono uniformemente distribuiti. È noto, tuttavia, che suoli naturali o agrari presentano delle eterogeneità causate principalmente dalla struttura fisica o macrostruttura del suolo, la quale influenza la distribuzione degli isotopi radioattivi e la loro accessibilità da parte dell’apparato radicale. L’obiettivo principale di questa tesi è stato quello di valutare l’effetto della distribuzione eterogenea degli isotopi radioattivi e della loro interrelazione spaziale (su un piano bidimensionale) con la distribuzione delle radici, sul trasporto di $^{54}\text{Mn}$, $^{57}\text{Co}$, $^{65}\text{Zn}$ e $^{134}\text{Cs}$ dal suolo alla pianta. L’analisi della distribuzione degli isotopi radioattivi nel profilo del suolo è stata effettuata mediante l’uso di sostanze coloranti che permettono la visualizzazione delle zone lungo le quali, data la presenza di macropori, l’acqua e soluti fluiscono in maniera preferenziale (zone di flusso preferenziale, ZFP). $^{54}\text{Mn}$, $^{57}\text{Co}$, $^{65}\text{Zn}$ e $^{134}\text{Cs}$ sono stati distribuiti sulla superficie di due suoli agrari non arati situati in Svizzera. Il mais è stato utilizzato come pianta modello. La distribuzione delle radici nel profilo del suolo e la loro interrelazione su un piano bidimensionale con le ZFP è stata effettuata mediante la tecnica delle mappe in-situ.

Le linee guide prescritte dall’Agenzia Internazionale per L’Energia Atomica per la determinazione del fattore di trasporto dal suolo alla pianta usato nei modelli matematici, prevedono esperimenti in serra e con suoli passati a setaccio e omogeneamente contaminati. Queste linee guida non rispecchiano la realtà dei suoli agrari e non possono pertanto descrivere in maniera adeguata il fenomeno.

L’effetto della struttura del suolo sul trasporto di $^{54}\text{Mn}$, $^{57}\text{Co}$, $^{65}\text{Zn}$ e $^{134}\text{Cs}$ nel mais è stato analizzato in un suolo agrario non arato. I risultati ottenuti sono stati messi a confronto con quelli ottenuti da piante cresciute in serra e nello stesso suolo passato a setaccio e omogeneamente contaminato prima di essere messo in vaso.

È stata osservata una maggior concentrazione degli isotopi radioattivi nelle ZFP rispetto alla matrice del suolo ed una moderata (10 al 15%) crescita preferenziale delle radici è stata osservata all’interno a nelle vicinanze delle ZFP. La quantità di $^{134}\text{Cs}$ e $^{57}\text{Co}$ misurata nelle parti aeree di mais coltivato in campo agrario è stata rispettivamente 2 volte e 10 volte maggiore della quantità misurata in piante cresciute in serra. Per $^{54}\text{Mn}$ e $^{65}\text{Zn}$ non è stata osservata nessuna differenza tra i due esperimenti.

La redistribuzione di $^{54}\text{Mn}$, $^{65}\text{Zn}$, $^{57}\text{Co}$ e $^{134}\text{Cs}$ nel profilo del suolo e il trasporto nelle parti aeree di mais è stato studiato in un secondo suolo agrario non arato e durante l’intero ciclo di crescita del mais.

È stata osservata una maggiore concentrazione degli isotopi radioattivi nelle ZFP rispetto alla matrice del suolo. Tuttavia, la quantità e la distribuzione stagionale delle precipitazioni, promuovendo un flusso convettivo verticale degli isotopi e la loro infiltrazione nella matrice del suolo, ha causato una diminuzione della concentrazione degli isotopi radioattivi nelle ZFP con il tempo. Una moderata (10 al
15%) crescita preferenziale delle radici è stata osservata all’interno a nelle vicinanze dell ZFP. Lo studio delle dinamiche temporali dell’assorbimento degli isotopi radioattivi durante un intero ciclo di crescita del mais ha dato risultati variabili a seconda del tipo di isotopo. L’assorbimento di $^{54}$Mn nelle parti aeree di mais è aumentato con il tempo, presentando dei valori significativamente più elevati ad ogni raccolta consecutiva. L’assorbimento di $^{57}$Co e $^{65}$Zn è stato più elevato in piante in fioritura rispetto a piante alla terza foglia, mentre è rimasto invariato tra la fioritura e la maturazione. Non è stato possibile misurare la quantità di $^{134}$Cs nelle piante alla terza foglia e alla maturazione. I risultati ottenuti nei due esperimenti effettuati su suoli agrari hanno dimostrato che solo una frazione (10-15%) dell’apparato radicale era localizzata nelle ZFP dove gli isotopi radioattivi erano maggiormente concentrati rispetto alla matrice del suolo. Questi risultati hanno suggerito che le radici presenti nelle ZFP erano responsabili nell’assorbimento degli isotopi radioattivi. Un esperimento a radici separate (split-root) è stato utilizzato per simulare la distribuzione eterogenea di $^{134}$Cs e delle radici. Una singola radice è stata immersa in una soluzione nutritiva contaminata con $^{134}$Cs, mentre il resto dell’apparato radicale è stato immerso in un adiacente contenitore riempito di soluzione nutritiva non contaminata. Pianta di cui l’intero apparato radicale è stato immerso in una soluzione nutritiva interamente contaminata con $^{134}$Cs sono state utilizzate come controllo. Due concentrazioni potassiche sono state utilizzate per verificare l’effetto della competizione tra Cs e K per i sistemi di trasporto a livello radicale. Un maggior assorbimento di Cs è stato ottenuto quando la concentrazione potassica della soluzione nutritiva era di 0.2mM. La singola radice, la cui sostanza secca rappresentava il 21% di quella dell’intero apparato radicale, ha assorbito il 50% della quantità assorbita dall’apparato radicale del controllo. La quantità traslocata alle parti aeree è stata del 47% rispetto al controllo. Questo effetto è stato circa tre volte inferiore quando la concentrazione potassica della soluzione nutritiva era di 1.05mM. Il modello matematico sviluppato da Barber-Claassen è stato utilizzato per predire l’assorbimento di $^{134}$Cs e K in piante alla terza foglia e in fioritura. Nelle piante in fioritura, i valori di assorbimento del $^{134}$Cs, erano di un ordine di grandezza superiore rispetto ai valori misurati, mentre quelli per il K erano dello stesso ordine di grandezza di quelli misurati ma due volte superiori. Non è stato possibile calcolare il valore di assorbimento del $^{134}$Cs e del K per le piante alla terza foglia a causa dell’impossibilità a determinare i parametri della cinetica di assorbimento secondo il modello di Michaelis-Menten. In seguito a questi risultati appare che il trasporto di isotopi radioattivi dal suolo alla pianta in suoli agrari sia dovuto a vari fattori, di cui l’effetto della struttura fisica del suolo sulla distribuzione degli isotopi radiattivi e delle radici rappresenta solo una parte. Altri processi sono coinvolti nelle dinamiche tra il suolo, la radice e gli isotopi radioattivi, quali: (i) condizioni ambientali (quantità e distribuzione stagionale delle precipitazioni), (ii) attività di microorganismi presenti nella rizosfera e di micorrizze, (iii) ridistribuzione degli isotopi all’interno della pianta, e (iv) competizione tra ioni.
General Introduction
Introduction

Soil-to-plant transfer of radionuclides: a field soil perspective

Radioactivity is the result of a natural change of an energetically unstable isotope of one element into an energetically stable isotope of a different element. All elements heavier than bismuth (Bi) exhibit natural radioactivity and thus can 'decay' into lighter elements (L'Annuziata, 1979). Radiation is a natural and ever-present part of our environment; in fact, the sun, the earth, and all living organisms emit radiation. In 1942, scientists were able to split atoms deliberately to release the energy contained in the nucleus, creating an unstable atom in the process, and thus giving birth to man-made nuclear energy (Bodansky, 2004). Since 1945 man-made radioactive elements have been released in the environment by three main sources:

- fallout products deposited after the nuclear weapon tests in the 1950s and 1960s
- controlled discharge of waste effluents from nuclear power plants
- accidental release (i.e. Chernobyl, Ukraine, April 1986)

Once the radioactive elements are introduced in the terrestrial environment, they can easily enter into the food chain by ground-water contamination after passage of the soil and vadose zone, by wet and dry deposition onto plant canopies, and by their uptake by roots and transport to the above-ground vegetation (Coughtrey, 1996). Thus, to limit and prevent contamination risks and health hazards it is important to make long-term predictions of the fate of radionuclides in the environment and to formulate realistic and effective remediation procedures. The radiological consequences from routine or accidental release of radionuclides into the terrestrial environment are usually evaluated with mathematical models (i.e. ECOSYS (Müller and Pröhl, 1993)).

In most of these models the uptake of radionuclides by plants is described by a transfer factor (TF). The TF is defined as the ratio of the radionuclide concentrations in vegetation and soil:

$$TF = \frac{C_p}{C_s}$$

where $C_p$ is the activity in Bq per kg of plant dry weight and $C_s$ is the activity in Bq per kg of soil dry weight (IAEA, 1994). Tabulated values for the transfer factor of various radionuclides for different plant-soil combinations are obtained under controlled conditions; i.e. in greenhouses where plants are grown on sieved and homogenously labelled soils. If experiments are carried out in the field, transfer factor values are standardized for a homogeneously contaminated layer of 20cm for crops and of 10cm for pasture (IAEA, 1994). The reason for the use of this extremely simplistic description of the transfer parameter in radiological assessment models is that it keeps model complexity down and it allows comparison between values obtained from different environments. Indeed, these models usually are designed to give conservative assessments (Peterson, 1995). However, soil-to-plant transfer factor of radiocaesium shows
ranges of up to three orders of magnitude even for individual soil-crop combinations (Frissel et al., 2002; Nisbet and Woodman, 2000). This type of approach to the study of the transfer of radionuclides form the soil to the plant, although widely used, has been recently criticized. Ehlken and Kirchner (2002) pointed out that using the TF concept is inadequate to describe the dynamics of soil-plant transfer processes because by its black-box definition it does not take into account the effects of soil chemical, biological and physical properties and of plant physiological processes, and it also does not allow to evaluate the influence of time-dependent variations of radionuclides accessibilities at the soil-root interface.

One of the most important differences between sieved soils and field soils is that the latter are characterized by a macro-structure and hence by the presence of macropores. Macropores are voids formed by soil fauna, decay of plant roots, wetting and drying processes, freeze-thaw cycles, or the erosive action of subsurface flow. Infiltration in the macroporous soil depends on the water content, intensity and amount of rainfall, hydraulic conductivity and surface contributing area (Weiler and Naef, 2003a). Water can flow into macropores from the soil surface or from a saturated or partially saturated soil layer (Weiler and Naef, 2003b). Macropore flow is more prevalent in no-till soils than in tilled soil due to the increased formation and preservation of biopores and a greater supply of water due to decreased surface runoff and evaporation (Shipitalo et al., 2000) (Figure 1). Lack of tillage allows surface residues to accumulate which favours the development of large populations of the surface-feeding earthworm *L. terrestris* (Edwards et al., 1990).

![Figure 1. Patterns of water movement in soil during the growing and dormant season as affected by tillage, based on studies conducted at the North Appalachian Experimental Watershed, OH, USA. (From Shipitalo et al., 2000).](image)

Furthermore, a lack of tillage allows macropores to persist longer and maintain continuity, potentially becoming more important pathways for rapid infiltration as the conductivity of the surrounding matrix decreases (Shipitalo and Edwards, 1996). Shipitalo et al (2000) reports that, based on long-term experiments on Luvisols at the North Appalachian Experimental Watershed, OH, USA, during the
growing season more water infiltrates in no-till soils than in tilled soils due to decreased surface runoff losses. During the dormant season (October to March), due to water soil conditions and lower intensity storms, a greater proportion of the percolation is through capillary-sized matrix porosity, and thus infiltration can be similar for no-till and tilled soil (Figure 1). Hence in field soils, macropores can induce a heterogeneous distribution of surface-applied radionuclides and affect root growth.

Distribution of surface-applied radionuclides in the soil profile

Surface-applied radionuclides are heterogeneously distributed in the soil profile due to structure-induced non-uniform water flow. It has been shown that highly sorbing solutes such as pesticides (Flury, 1996) and selected radionuclides (Albrecht et al., 2002; Bundt et al., 2000) accumulated within and around macroporous preferential flow pathways. The rapid and non-uniform transport of water and solutes allows solutes to bypass a large part of the potentially sorbing soil matrix or to move rapidly through and out of the rooting zone (Flury et al., 1994). The most important causes of preferential flow are the presence of macropores and other structural features, development of flow instabilities (i.e. fingering) caused by profile heterogeneities or water repellence and funnelling of flow due to the presence of sloping soil layers that redirect downward water flow (Simunek et al., 2003). Furthermore, the remobilization and transport of radionuclides to greater soil depths can occur through facilitated colloid transport. Because of their small size, large surface area, and charge properties, mobile colloids (mineral and organic) can potentially have greater metal sorption affinity than the soil matrix (Karathanasis, 1999). Thus a high affinity for sorption to colloidal minerals or organic matter would no longer guarantee a low mobility of highly sorptive solutes as radionuclides in soils. However, facilitated transport can only become a relevant mechanism when a sufficient number of preferential pathways are active (Zehe and Fluhler, 2001).

Distribution of roots

Roots grow in soils in two ways: in existing voids, or by penetrating the soil matrix, thus creating new macropores (Schoonderbeek and Schoute, 1994). In no-till soils the formation and presence of vertically oriented biopores that are suitable for preferential root growth counteract the possible negative effects of increased bulk density on root development (Chassot et al., 2001). Stewart et al (1999) showed that in uncultivated and coarsely structured black vertisols up to 80% of the root system was located within macropores or closely associated with them. Bundt et al. (2000) also observed a significantly higher root biomass of various tree species in the preferential flow paths than in the matrix of an acid brown forest soil. Van Noordwijk et al. (1993) developed a statistical test for synlocation of roots and cracks on the basis of measured root densities as a function of the distance to the nearest crack and found a significant increase in root density (by a factor of 2) close to the crack. Finally, Pierret et al. (1999) and Pankhurst et
al. (2002) demonstrated the existence of an environment around soil macropores that is chemically and microbiologically different from the bulk soil, supporting active microbial populations that differ quantitatively and functionally to those present in the bulk soil.

Although the effect of macropores on the distribution of surface-applied contaminants has been widely investigated and it is known that roots distribute unevenly in field soil, the soil-to-plant transfer of contaminants is still studied primarily in homogenous environments of laboratory or greenhouse systems. We postulated that the uptake of radionuclides in field soils is influenced by the heterogeneous distribution of surface-applied radionuclides and their spatial interactions with the roots.

The project was conducted with an interdisciplinary perspective in the collaboration with the group of Terrestrial Ecology (ETH, Zürich) and the group of Transfert sol-plante et cycle des éléments minéraux dans les écosystèmes cultivés (INRA, Bordeaux).

**Objective, hypotheses and structure of the thesis**

The objective of this dissertation was to evaluate the influence of the heterogeneous distribution of surface-applied radionuclides and their spatial relation with roots on the soil-to-plant transfer of four radionuclides (\(^{54}\text{Mn}, ^{57}\text{Co}, ^{65}\text{Zn}\) and \(^{134}\text{Cs}\)).

The thesis is structured into four main chapters. In the first chapter we questioned the adequacy of the Transfer Factor (TF) determined according to the standard protocol of IAEA (1994) for predicting the radionuclides uptake by plants in field soils. Standard experiments are carried out in the greenhouse with plants grown in sieved and homogeneously contaminated soils. We analyzed the effect of the soil macrostructure on the displacement of surface-applied radionuclides, their sorption onto soil solids, the degree of soil exploration by roots, and their overall influence on the recovery of \(^{54}\text{Mn}, ^{57}\text{Co}, ^{65}\text{Zn}\) and \(^{134}\text{Cs}\) by maize (Zea Mays) grown on an un-tilled agricultural soil. In order to evaluate the results of the field experiment in relation to a controlled experiment we studied the recovery of the four radionuclides by maize grown also in the greenhouse and on the same soil that was sieved and homogeneously labelled before being repacked in pots. We postulate that plants grown under field conditions will take up more radionuclides than plants grown under controlled conditions in the same, sieved and homogeneously radio-labelled soil, because under field conditions surface-applied radionuclides accumulate predominantly in the well rooted regions within and near water flow paths.

In the second chapter we analyzed the temporal variations of radionuclide accessibility by roots occurring at the soil-root interface. We studied the redistribution of the surface-applied radionuclides (same as those mentioned above) in the soil profile and their uptake by maize grown on an untilled agricultural soil during a growing season. We postulated that plant uptake increases during the plant growth season.
because the root system develops and explores an increasing fraction of the soil volume, reaching more frequently the niches of radionuclide enrichment.

Results obtained in the first and second chapter have shown that in this soil only a small fraction (10 to 15%) of the root system is located in the preferential flow pathways where radionuclides are concentrated in respect to the soil matrix. This suggests that the roots located in these areas were responsible for the uptake of radionuclides. Hence, the aim of the third chapter was to assess to what extent a small fraction of the root system, growing in areas of $^{134}$Cs enrichment, may contribute to the total uptake and translocation of $^{134}$Cs in relation to its uptake and translocation by the whole root system growing in an homogeneously contaminated medium. We postulated that the uptake of $^{134}$Cs by a single root growing in a $^{134}$Cs enriched area accounts for most of the uptake relative to that of the whole root system growing in a homogeneously contaminated medium. In the fourth chapter we analyzed the possibility of using the Barber-Claassen model for predicting the uptake of $^{134}$Cs by maize. The model has been successfully tested in agricultural systems for various nutrients (Barber, 1984), heavy metals (Mullins et al, 1986) and recently for $^{134}$Cs in pea plants (Roca-Jove and Vallejo-Calzada, 2000). In contrast to the work of Roca-Jove and Vallejo-Calzada (2000), we have directly measured all the necessary model parameters by carrying out specific experiments.
Experimental approach

The analysis of the distribution of surface-applied radionuclides was carried out using dye tracers which allowed the visualization of the structure-induced water flow paths. The behaviour of tracers with different chemical properties can be examined under field conditions using them as observable surrogates compounds that behave similarly to certain pollutants as pesticides and radionuclides (Aeby et al., 2001). Both radionuclides and dye tracers were applied in water solution on the soil surface. The amount of water applied corresponded to a heavy thunderstorm shower. This type of irrigation was carried out in order to induce an infiltration of water within the areas of lower porosity (macropores) and in the preferential flow paths, hence allowing us to analyze the hypothesis that heterogeneous distribution of radionuclides and roots can influence their uptake. This also allowed us to study the migration of surface-applied radionuclides and dye tracers at greater soil depths. The distribution of root and their relation with the localization of radionuclides enrichment was carried out by in-situ mapping technique. $^{54}\text{Mn}$, $^{57}\text{Co}$, $^{65}\text{Zn}$, and $^{134}\text{Cs}$ were used to study the uptake of contaminants by maize which was used as a model plant.

Why using $^{54}\text{Mn}$, $^{57}\text{Co}$, $^{65}\text{Zn}$, and $^{134}\text{Cs}$?

We have chosen these man-made $\gamma$-emitter radionuclides because of their absence in natural system. They can also be readily measured with $\gamma$-spectroscopy at very low activities, allowing field application below the Swiss Safety standard (Bewilligungsgrenze der Strahlenschutzverordnung). Furthermore, their different chemical and physiological properties allowed us to make a more consistent and wider applicable evaluation of the influence of the physical and chemical soil heterogeneities on the soil-to-plant transfer of radionuclides.

Chemical and physiological properties of $^{54}\text{Mn}$

Mn is a transition metal which exhibits three possible oxidation states in soils $+2$, $+3$ and $+4$. $\text{Mn}^{2+}$ is the only stable form in soil solution. $\text{Mn}^{3+}$ and $\text{Mn}^{4+}$ are stable only in the solid phase of the soil, where they form insoluble oxide and hydroxide minerals of variable structure and oxidation state ($\text{MnO}_2$, $\text{MnOOH}$, $\text{Mn}_3\text{O}_4$) (Mcbride, 1989). Mn solubility is controlled by the redox potential and pH of the soil. The $\text{Mn}^{2+}$ is a very soluble species in water, forming hydroxide and carbonate precipitates only at high pH ($>7$). Low pH or low $E_0$ favours the reduction of insoluble Mn oxides and an increased solubility of $\text{Mn}^{2+}$. As the pH is raised above 6 in soils, $\text{Mn}^{2+}$ solubility decreases because it bonds with organic matter, oxides, carbonates and silicates (Mcbride, 1989). Small changes in the $E_0$-pH conditions can be very important in the Mn content of the soil solution; hence Mn solubility within any particular soil can fluctuate tremendously over time. The mobility of Mn defies classification because it is extremely sensitive to soil conditions (acidity, wetness, biological activity) (Kabata-Pendias, 2001a).
The most important Mn function in higher plants is related to the oxidation-reduction process. Mn acts as a cofactor activating about 35 different enzymes that catalyze oxidation-reaction, decarboxylation, and hydrolytic reactions. Mn appears to participate in the O₂-evolving system of photosynthesis and also plays a basic role in the photosynthetic electron transport system (Marschner, 1995). Mn uptake is metabolically controlled, apparently in a way similar to that of other divalent cation species such as Mg²⁺ and Ca²⁺. Passive transport absorption of Mn is also likely to occur, especially in the high and toxic range of this metal in solution (Kabata-Pendias, 2001a).

In most plants Mn concentrations are highest within the roots, followed by the leaves and then the stem, flowers and seeds (Pearson and Rengel, 1994). Mn is suggested to be remobilized out of the root via xylem (Loneragan, 1988). Pearson and Rengel (1994) studying the remobilization of Mn in wheat reported that the extent to which Mn is mobile within the phloem is limited.

Microbiological soil activity is also known to be largely responsible for the oxidation and reduction on Mn compounds, as well as for the formation on Mn concretions. Many members of the bacterial and fugal genera Bacillus, Pseudomonas, Arthrobacter, Steroptomycetes, Aspergillus, can mediate Mn reduction via abiotic reactions with their metabolic end product and/or enzymatic reduction using Mn⁴⁺ as a terminal electron acceptor and thus increasing Mn availability for plant (Posta et al., 1994). These processes are likely to occur at acid pH and appropriate Eₚ in soils. Some micro-organisms, on the other hand, can precipitate Mn by oxidizing Mn²⁺ to Mn³⁺ and Mn⁴⁺ or stimulating the precipitation of carbonates, sulfides, etc. (Kabata-Pendias, 2001a).

Posta et al. (1994) have also shown that the inoculation with vesicular arbuscular mycorrhizal fungi in maize plants decreased the Mn concentration in shoots almost to that of the control treatment. Mycorrhizal infection decreased not only Mn concentration in the shoots but also root dry weight and induced quantitative and qualitative changes in the rhizosphere micro-organisms.

**Chemical and physiological properties of ⁵⁷Co**

Cobalt is a transition metal which occurs in two oxidation states in soil, +2 and +3, but Co²⁺ is the dominant form in soil solution. Co associates preferentially with Fe and Mn oxides because of chemisorption and co-precipitation. On Mn oxides, Co²⁺ is oxidized and strongly bound as Co³⁺. Soil pH is the most important factor determining the availability of Co. Co is relatively mobile in oxidizing acid environment (Mcbride, 1989). As the soil pH is raised above 7, Co solubility decreases because of increased chemisorption on oxides and silicate clays, complexation by organic matter, and possibly precipitation of Co(OH)₂ (McLaren et al., 1986). Organic matter complexes with Co²⁺ are fairly labile, so that organically bound Co²⁺ can become bioavailable (Mcbride, 1989). Soil drainage status has a major influence on the amount of extractable Co available for plant uptake. In poorly drained soils the amount of
extractable Co is generally greater than in adjoining areas which are well drained, and plant uptake is significantly increased (Smith and Paterson, 1995).

There is no evidence that Co has any direct role in the metabolism of higher plants (Marschner, 1995), although there are evidences of a favourable effect of Co on plant growth (Moreno-Caselles et al., 1997). Beneficial effects of low Co concentrations on plant metabolism are not yet fully understood. Presumably, several effects are cross-linked with interactions with other trace metals (Kabata-Pendias, 2001b).

Co is known to be required by Rhizobium species as the metal component of the coenzyme cobalamin (vitamin B_{12}) in root nodules of leguminous plants and by some species of nitrogen-fixing blue-green algae for symbiotic nitrogen fixation. The uptake of Co by plants has been investigated in a number of studies and is known to be affected by several factors such as temperature, pH, and light/dark (Liu et al., 1998).

The question of whether there are specific transporters for trace metals such as Co, or whether Co uptake occurs via a relatively non-specific divalent metal transport, remains unanswered (Liu et al., 1998). As reported by Marschner and Dell (1994) and by Suzuki et al. (2001) Co and Mn are little transported by arbuscular mycorrhizal fungi.

**Chemical and physiological properties of 65Zn**

The solubilization of Zn minerals during weathering produces mobile Zn^{2+}, especially in acid, oxidizing environments. The 2⁺ oxidation state is the only one possible in the soil. In acid (~pH 5), aerobic conditions the adsorption of Zn^{2+} can be reduced by competing cations and this results in easy mobilization. At higher pH values (~7), chemisorption on oxides and alluminosilicates and complexation with humus lower the solubility of Zn^{2+} markedly (Kiekens, 1995). Consequently, Zn mobility in neutral soils is very low. At higher pH values, while an increase of organic compounds in soil solution becomes more evident, Zn-organic complexes may also account for the solubility of this metal (Mcbride, 1989). In the reducing environment of flooded soils, release of Zn^{2+} from dissolving Fe oxides may initially increase availability.

Acid leaching is very active in Zn mobilization because of increased solubility and formation of soluble complexes with organic ligands. Karathanasis (1999) investigating the potential role of water-dispersible soil colloids in transporting Zn and Cu through undisturbed soil column obtained from six different silt loamy soils, have shown that low ionic strength subsurface environments, which enhance solid-phase dispersion, moderately high pH and OC levels may significantly enhance Zn transport.

Zn plays an essential metabolic role in the plant, of which the most significant is its activity as a component of a variety of enzymes, such as dehydrogenase, proteinase, peptidase, and phosphoydrolases. Basic Zn functions in plants are related to metabolism of carbohydrates, proteins, phosphate and also...
auxins, RNA, and ribosome formation (Kabata-Pendias, 2001c). Zn is involved also in cellular processes of protein synthesis and membrane stability (Marschner, 1995).

It appears that root Zn$^{2+}$ uptake is mediated by two different transport systems: a high-velocity, low-affinity system (Km= 2-5 μM) that operates at relatively high soil Zn concentrations, and a low-velocity, high-affinity system (Km= 0.6-2 nM), that probably is the dominant transport system under low soil Zn conditions. The high-affinity transporter follows Michaelis-Menten kinetics (Hacisalihoglu and Kochian, 2003). The distribution of Zn between root and shoot is dependent on Zn supply. An adequate Zn supply leads to greater proportion of Zn in the shoot, while toxic Zn supply leads to an accumulation within the roots (Pearson and Rengel, 1994). Similarly, Zn remobilization is dependent upon the demand of Zn elsewhere within the plant.

Zn availability to plants also depends on the capacity of different species to mobilize soil Zn via a range of rhizosphere processes. The major root-induced processes are: (1) rhizosphere acidification or decrease in redox potential and consequent dissolution of soil Zn-bearing phases (Marschner et al., 1986); (2) exudates of Zn-chelating phytosiderophores (Graham and Stangoulis, 2003), organic ligands and consequent complexation of metals in the soil solution (Kabata-Pendias, 2001c).

It has been shown that arbuscular mycorrhizal fungi (AMF) are able to take up and transport significant amounts of trace elements (Zn) to roots (Bürkert and Robson, 1994). The smaller diameter (μm) of AMF hyphae and their capacity to extend into zones remote from the root system allow the exploration of areas which are spatially and physically inaccessible to plant roots (Jansa et al., 2003).

Chemical and physiological properties of $^{134}$Cs

Cs is the second heaviest element of the Group I alkali metals and displays a high degree of chemical similarity with K in particular (Bowen, 1979). In solution, Cs exists predominantly as a monovalent cation.

It is now generally recognized that specific adsorption of radiocaesium is associated with the presence, even in small quantity, of micaceous clay minerals (illite and vermiculite). The interlayer space of these phyllosilicates exhibits areas formed by the juxtaposition of non-expandable and hydrated zones. In these clays the high negative charge generated between layers is neutralized by cations of low hydration energy, basically K. These cations lose their hydration water and induce collapse of two adjacent layers, thus leading to a very stable structure. At the edges of the two clay layers, weathering occurs with the subsequent release of K and interlayer expansion. Such areas are called wedge zones and their associated sites are the frayed edge sites (FES). The FES contributes to less then 2% of the overall CEC. They selectively sorb poorly hydrated alkaline cations K, Rb, Cs as well as NH$_4^+$ ion (Maes et al., 1999).
The low hydration energy is the major factor explaining the specific sorption of trace Cs by micaceous clay minerals. The adsorption of Cs by micaceous phyllosilicates can be described in terms of at least three kinds of sites (Delvaux et al., 2001) exhibiting a decreasing selectivity for poorly hydrated cations:

1. the *frayed edge sites* (FES), distinguishable in:
   a. highly selective sites (05% of the total FES)
   b. intermediate sites (2-5% of the total FES)
   c. low-selectivity sites (97-98% of the total FES)
2. the *hydrated interlayer sites* in which the hydrated cations can be exchanged with poorly hydrated cations, this ion exchange can cause the collapse of the interlayer space;
3. the *regular exchange sites* (RES) are the planar sites located on the external surfaces of the phyllosilicates and regular ion exchange processes occur on such sites.

Among soil colloids organic matter weakly retains trace Cs in soil. Indeed, the retention of radiocaesium by soil organic matter is reported to involve chiefly electrostatic binding, even if chelating agents are present. Only in organic soils with more then 95% organic matter content and negligible clay contents does adsorption occur mostly in non-specific sites (Regular Exchange Sites) (Rigol et al., 2002). Cs does not hydrolyze readily; hence over a wide pH range it is present as Cs\(^+\). The pH of the system is, therefore, only important in so far as it affects the exchange properties of the substrate not because of any effect on Cs speciation (Cornell, 1993).

Although there is no known role of Cs\(^+\) in plant nutrition (Marschner, 1995), Cs can be transferred into plants due to its physiological similarity with K (Shaw and Bell, 1991). At low external K concentration (<12mg/l), K uptake is dominated by a carrier-mediated high-affinity transport (H\(^+\)/K\(^+\) co-transporter) system, which has low selectivity between K and Cs (Véry and Sentenac, 2003), therefore Cs can effectively compete with K for uptake sites. Conversely, at higher external concentration of K (often >12-40mg/l), the electrochemical gradient is diminished or reversed, and uptake has dominantly channel-like properties. This low-affinity transport system highly discriminates against Cs (Sacchi et al., 1997).

Little is known on the mobility of the absorbed Cs within the plant. Recently, Feller et al. (2000) have shown that in steam-girdling experiments where the transport of \(^{134}\)Cs in the xylem sap of detached wheat shoot was stopped \(^{134}\)Cs was loaded into the phloem during acropetal transport.

Effects of rhizosphere process on the remobilization of Cs are poorly understood. Delvaux et al. (2000) carrying out experiments in several soil horizon with widely varying properties and placed into close contact with an active rhizosphere of ray grass for 4 days, have shown that \(^{137}\)Cs rhizospheric mobilization was strongly correlated with K depletion in the rhizosphere.
Arbuscular mycorrhizal fungi are highly unlikely to play any significant role in plant uptake of radiocaesium through the process of fungal transport (Joner et al., 2004).

Maize as a model plant
Maize (Zea mays L.) has an important agronomic relevance being the third most planted field crop (after wheat and rice). Maize is a fast growing and deep rooting plant, being suited for short term-laboratory experiment and allowing a good characterization of root distribution at greater soil depth. The root system of maize is well characterized and is made up of seminal roots, including the radicle and seminal adventitious roots appearing on the scutellar node, and adventitious nodal roots appearing on the stem at the bottom part of successive phytomers (Pellerin and Pagès, 1994) (Figure 2).

Figure 2. Structure of the bottom part of the maize plant (Pellerin and Pagès, 1994).
54Mn, 57Co, 65Zn, and 134Cs uptake by maize grown under field and pot conditions

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Abstract
Risk assessment models of soil-to-plant transfer of contaminants rely on the assumption that soils are homogeneously contaminated. We compared the uptake of $^{54}$Mn, $^{57}$Co, $^{65}$Zn and $^{134}$Cs by corn (Zea Mays) grown on an un-tilled agricultural soil with the uptake by maize grown on the same soil that was sieved and homogeneously labelled before being repacked in pots. A significantly higher recovery of $^{57}$Co (2-fold) and $^{134}$Cs (10-fold) was observed in the plants grown in the field soil, whereas no differences in the recovery of $^{54}$Mn and $^{65}$Zn between the two experiments was detected. The study of the interrelation between roots and radionuclide distribution as well as availability indicated that various factors can affect the uptake of surface applied radioactive elements, such as (i) the heterogeneous distribution of radionuclides due to the structure-induced heterogeneous water flow, (ii) the accessibility of trace elements and competing ions sorbed within aggregates and other fine porous structures, and (iii) the uptake of radionuclides by arbuscular mycorrhizae hyphae and root, and their transfer to the aerial parts.
Introduction

The radiological impact of radionuclides released to the terrestrial environment is usually predicted with mathematical models. In such models the transfer of radionuclides from soil to the plant is described with the transfer factor (TF) (IAEA, 1994). The transfer factor is defined as the ratio of the radionuclide concentrations in vegetation and soil. Transfer factors (TF) are determined either in greenhouses or field studies. The greenhouse experiments are carried out with plants grown in pots filled with sieved soil that has been homogeneously labelled with radionuclides. The field experiments are mostly making use of the fallout products naturally incorporated in the field soil by infiltration, bioturbation and other mixing processes. The fallout products are the radionuclides deposited after the nuclear weapon tests in the 1950s and 1960s, after the Chernobyl accident in 1986, and after controlled release from nuclear installations (IAEA, 1994). In the IAEA (1994) handbook of parameters values for the prediction of radionuclide transfer, TF values are standardized for a homogenously contaminated soil layer of 20cm for crops and of 10cm for pasture.

Ehlken & Kirchner (2002) have recently criticized the use of the TF to study the transfer of radionuclides from the soil to the plant. These authors point out that the TF integrates various soil chemical, biological, hydrological and physical properties and plant physiological processes, each of which showing its own variability and being influenced by external factors. For instance, the soil macro-structure influences the distribution of water and solutes in the soil. Preferential flow (rapid transport of water and solutes) allows solutes to bypass a large part of the potentially sorbing soil matrix or to move rapidly through and out of the root zone (Flury et al., 1994). Short-term laboratory and field experiments showed that preferential flow is the predominant transport mechanism which dramatically enhances the mobility of highly sorbing solutes such as pesticides (Flury, 1996; Stamm et al., 1998), fertilizers especially phosphate (Stamm et al., 1998), and radionuclides (Albrecht et al., 2002; Bundt et al., 2000).

The soil macro-structure can also strongly affect soil chemical processes. Sinaj et al. (2002) observed an increased P availability in the preferential flow paths (PFP) of soils that had been fertilized with P and that were regularly irrigated. Furthermore, Sinaj et al. (1997 and 1999) showed that the rate of transfer of $^{33}$P and $^{65}$Zn from the solution to the solid phase of the soil was affected by the state of dispersion of the soil particles, and Bühler et al. (2003) observed that $^{33}$P exchanged more rapidly against $^{31}$P in a stirred suspension at 1:10 soil to water ratio then in a pot experiment at 0.25-0.30 m$^3$ m$^{-3}$ water content and with little disturbed macro-structure.

The soil macro-structure also affects the root morphology and distribution of field grown plants. Bundt et al. (2000) demonstrated a relationship between soil structure and roots, observing a higher root distribution in the vicinity of preferential flow paths. Stewart et al. (1999) found root clustering at short distances (1-10mm) from macropores and more randomly distributed roots at longer distances. Pierret et
al. (1999) showed that the preferential location of roots in and around soil macropores provides a local environment that is likely to have higher levels of biological activity. Altogether these results suggest that soil macro-structure can affect the displacement of surface-applied radionuclides, their sorption onto soil particles, and the degree of soil exploration by roots. As a consequence they imply that soil structure might indirectly affect radionuclide uptake by the plant.

The aim of this work was to compare the uptake of four radionuclides (\(^{54}\text{Mn}, {^{57}\text{Co}, {^{65}\text{Zn and } {^{134}\text{Cs}}}\)) by maize grown in a field soil where the radionuclides were applied onto the soil surface with the uptake by maize grown under controlled conditions in a greenhouse on the same soil that was sieved and homogeneously labelled before being repacked in pots. We postulated that plants grown under field conditions will take up more radionuclides than plants grown under controlled conditions in the same, sieved and homogenously radio-labelled soil, because under field conditions surface-applied radionuclides accumulate in the well rooted regions within and near water flow paths. Plant growth, radionuclide and nutrient uptake, and root weight density were analyzed both, in the field and in the pot experiment. In the field experiment radionuclide displacement in the soil profile and the interrelation between roots and flow paths distribution patterns were also analyzed. The effect of crushing the aggregates and sieving on the availability of radionuclides, their stable elements and potassium was studied in a batch experiment.

**Materials & Methods**

**Field experiment**

*Soil characteristic:* The study was carried out on a field site located at Eschikon (47° 27’ N, 8° 41’ E, and 550 m elevation) 20 km east of Zurich, Switzerland on a clayey loamy soil (Eutrochrept). Selected soil chemical and physical properties are given in Table 1.1. The field has a slope of around 1%. The experimental plot has been under grassland and has not been ploughed for the last 30 years. Three horizons were identified: A\(_h\) horizon (0-20cm) rich in organic matter, B (20-40cm) characterized by a higher bulk density than the A\(_h\) horizon, and a BC horizon (>40cm) above the bed rock (moraine), compacted, brighter in colour and stony with a skeleton content up to 50% by volume.

*Experimental design of the field study:* The three plots were separated 15 cm from each other. The plots were 5.6m x 1.05m. The plant density was 8.3 plants m\(^{-2}\), with 80 cm between rows and 15 cm between plants in the row. The radionuclide solution was applied on the central area (2.4m x 0.45m) of each plot encompassing the central three rows and three plants per row. Plants were sampled in these central plots only. The area outside the central plots was used to minimize edge effects (Figure 1.1).
Table 1.1 Selected soil chemical and physical properties of the field site.

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Skeleton</th>
<th>Sand( \uparrow )</th>
<th>Silt( \uparrow )</th>
<th>Clay( \uparrow )</th>
<th>Bulk density( \uparrow )</th>
<th>Organic matter( \uparrow )</th>
<th>Porosity( \uparrow )</th>
<th>pH</th>
<th>CEC( \dagger \uparrow )</th>
<th>Mn( \dagger )</th>
<th>Zn( \dagger )</th>
<th>Co( \dagger )</th>
<th>Cs( \dagger )</th>
<th>K( \dagger )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cm)</td>
<td>(g kg(^{-1}))</td>
<td>(g kg(^{-1}))</td>
<td>(g m(^{-3}))</td>
<td>(g kg(^{-1}))</td>
<td>(g kg(^{-1}))</td>
<td>(cmolc kg(^{-1}))</td>
<td></td>
<td>(g kg(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A (_h)</td>
<td>(0-20)</td>
<td>413</td>
<td>320</td>
<td>267</td>
<td>1.1</td>
<td>57</td>
<td>0.53</td>
<td>7.4</td>
<td>18.3</td>
<td>771±2</td>
<td>69±1.1</td>
<td>10.2±2.3</td>
<td>3±1</td>
<td>10374±29</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>(20-40 )</td>
<td>343</td>
<td>357</td>
<td>300</td>
<td>1.5</td>
<td>16</td>
<td>0.41</td>
<td>n.d</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>BC</td>
<td>(&gt;40 )</td>
<td>500</td>
<td>318</td>
<td>44</td>
<td>279</td>
<td>1.7</td>
<td>2</td>
<td>0.35</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

\( \uparrow \) Based on sedigraph analysis, after 6 min of ultrasound treatment in 0.1% Na-hexametaphosphate.

\( \dagger \) Schweizerische Referenzmethoden der Eidgenössischen landwirtschaftlichen Forschungsanstalten (1999).

\( \dagger \uparrow \) CEC: cation-exchange capacity (Thomas, 1982).

\( \dagger \) X-ray fluorescence spectrometry.

n.d.: not determined
Radionuclides uptake by maize grown under field and pot conditions

Figure 1.1 Plot design. (A) Overview of the layout in the field experiment. x = plants; grey areas = traced with radionuclides; white areas = buffer to minimize edge effects; (B) Sampling scheme in traced area; black horizontal lines = position of the vertical profiles; dashed area = location of the horizontal planes; while white circles = auger sampling.

Radiotracer application: On July 23, 2001, a solution containing $^{54}$Mn, $^{65}$Zn, $^{57}$Co and $^{134}$Cs, all in chloride form diluted in a weakly acid solution, was applied onto the soil surface of the three central plots (Table 1.2). Radionuclides were purchased at Amersham® (Germany). The solution was applied manually, using a watering can with a sprinkling bar fixed at the end of the spout. To avoid surface ponding the solution was applied in 12 slopes of equal amounts of 10L in a period of 6h. The 120L of solution contained 71, 33, 9.5 and 37kBq mL$^{-1}$ of $^{54}$Mn, $^{57}$Co, $^{65}$Zn and $^{134}$Cs respectively; dissolved in Osmosis I water of an electrical conductivity of 17μS cm$^{-1}$ at 25°C. To avoid lateral infiltration toward the rows, a similar amount of Osmosis I water was applied at the same rate on the buffer area outside the central plots.
Table 1.2 Characteristics and quantities of the four ($^{54}$Mn, $^{65}$Zn, $^{57}$Co and $^{134}$Cs) radionuclides applied (a) to the soil surface of an untilled agricultural soil (field experiment) and (b) added to the same homogenized soil (pot experiment).

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Chemical Form</th>
<th>Specific activity (MBq mg$^{-1}$)</th>
<th>Total activity applied (kBq)</th>
<th>Activity applied (kBq m$^{-2}$)</th>
<th>Activity applied (kBq kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Field experiment</td>
<td>Pot experiment</td>
<td>Field experiment</td>
<td>Pot experiment</td>
<td></td>
</tr>
<tr>
<td>$^{54}$Mn</td>
<td>$^{54}$MnCl$_2$</td>
<td>74</td>
<td>473</td>
<td>414</td>
<td>146</td>
</tr>
<tr>
<td>$^{65}$Zn</td>
<td>$^{65}$ZnCl$_2$</td>
<td>13</td>
<td>71</td>
<td>72</td>
<td>22</td>
</tr>
<tr>
<td>$^{57}$Co</td>
<td>$^{57}$CoCl$_2$</td>
<td>50</td>
<td>794</td>
<td>684</td>
<td>245</td>
</tr>
<tr>
<td>$^{134}$Cs</td>
<td>$^{134}$CsCl</td>
<td>37</td>
<td>45</td>
<td>90</td>
<td>14</td>
</tr>
</tbody>
</table>

Plants: On May 20, 2001, the grass was burnt and a solution of 1% of Glyphosate (Roundup) was applied at the rate of 0.42ml m$^{-2}$. Furrows were manually prepared and maize (Zea Mays L. cv. Corso) was sown on July 22, 2001. After sowing, the soil was fertilized with 11g N m$^{-2}$ as NH$_4$NO$_3$, 4.1 g P m$^{-2}$ as KH$_2$PO$_4$ and 12.5 g m$^{-2}$ as KCl, in solid form as recommended for maize production in Switzerland. Fertilizers were manually applied in a solid form. The plants were not irrigated to reduce radiotracer mobility during the experiment. During the growing period plant height was measured regularly. A weather station placed 60m from the site, recorded air and soil temperatures, rainfall and solar radiation (Table 1.3).

On September 17, 2001, after two months of growth when plants had reached the flowering stage, the shoots were harvested by cutting the stem 1cm above the soil level, and then washed with tap water, chopped and oven-dried at 105°C for 24h. The whole shoot was used for plant analyses. The dry weight was determined and the dry material milled to powder. A portion of the sample was used to measure Mn, Zn, Co, Cs, K, N and P while the remaining part was weighted and transferred into calibrated γ-spectrometry containers. Nitrogen was extracted by using the Kjeldahl method, whereas the other elements, after ignition of the sample at 500°C for 1h, were extracted with 2ml of 0.1M HCl solution, filtered through a Whatman-40 ashless cellulose filter and diluted to 50ml with deionised water. The concentration of P in the diluted solution was measured by colorimetry using the method of Murphy and Riley (1962) while the concentration of cations was measured by ICP-MS.

Table 1.3 Meteorological data recorded during the growing period of maize plants in the field experiment in 2001.

<table>
<thead>
<tr>
<th>Rainfall (mm)</th>
<th>Soil temperature</th>
<th>Air temperature</th>
<th>Solar Radiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-5cm</td>
<td>5-10cm</td>
<td>10-20cm</td>
</tr>
<tr>
<td>July</td>
<td>46.2</td>
<td>20.1</td>
<td>19.7</td>
</tr>
<tr>
<td>August</td>
<td>117.2</td>
<td>20.3</td>
<td>20.2</td>
</tr>
<tr>
<td>September</td>
<td>137.6</td>
<td>14.8</td>
<td>15.2</td>
</tr>
</tbody>
</table>
Dye tracer application and analysis of radionuclide distribution on soil vertical profiles: The dye tracer Brilliant Blue FCF was applied immediately after harvest onto the soil surface in order to visualize the preferential flow paths (stained areas) from the soil matrix (unstained areas). In delineated depths of 0-5, 5-15 and 15-35, samples were taken with a small spatula from the stained regions, representing preferential flow paths, and from the unstained soil matrix. The results on the correlation between the dye tracer and the radionuclide activities are described by Penfield et al. (2002).

Mapping root and flow path distribution: On each plot two horizontal planes were prepared above each other at depth of 0.2m and of 0.4m, respectively. They had an area of 0.45m parallel to the row and 0.8m perpendicular to the row (Figure 1.1). On the two horizontal planes the occurrence of roots intersecting the plane of observation was mapped with a filt-tip pen onto polythene sheets with a grid of 5cm x 5cm to systematically locate root occurrence (Tardieu and Manichon, 1986). A second sheet was used to trace the preferential flow paths by drawing the contours of the stained areas. Root maps were scanned and digitized using Scion Image (version beta 4.02) for recording the x,y coordinates of the individual roots. Maps of the flow paths areas were scanned and digitized using IDL (version 4.01 of Interactive Data Language, Research Systems Inc., Co) to determine the dye coverage (fraction of stained area in relation to the exposed plane). The corners of the grid borders were used as reference points to superimpose the flow path and root map. The number of roots occurring within the preferential flow paths was determined. All three plots displayed similar root and stained area patterns and thus only the maps obtained from the middle plot are shown as example (Figure 1.3).

Root weight density: In each plot six cylindrical cores (20cm long and 5cm in diameter) were sampled at two depths (0-20cm and 20-40cm) using a hand-hold auger. To obtain a realistic representation of the root distribution in relation to the plant position, four samples were taken at 5cm from the plant row and at 40cm from the plant row, where root density tends to decrease (Figure 1.1). Soil was rinsed off from the root samples using the hydro pneumatic elutriation system, (Gillson’s Inc.) developed by Smucker et al. (1982). Roots were freeze-dried for 48h. Root dry weight was determined and the root weight density (g cm\(^{-3}\)) calculated. Measurements of the radionuclide activities in root samples obtained from the field experiment were erroneous and unreliable due to the fine soil particle adhering on the root epidermis.

Pot experiment
Untreated soil was collected from the area surrounding the field experiment. The quantity of soil taken from the field site corresponded to an area of 0.72m\(^2\) (1.6m x 0.45m) and a depth of 20cm. The soil was air-dried at 24°C for one week. Stones and plant debris were separated and the soil was sieved at 6mm. The sieved soil was divided into two portions of 90kg and each portion was spiked with a solution containing the four radionuclides used in the field experiment (activities are shown in Table 1.2) and 35L of Osmosis I water. This amount of water was chosen to bring the soil water content to a value of 0.28 m\(^3\).
m$^{-3}$ which corresponds to the water content of the field soil measured after plant harvest in samples taken from 0 to 20cm depth (average value). To obtain a homogeneous activity, each soil portion was mixed with the solution for 3h in a rotating concrete mixer. The labelled soil was divided in six equal parts of 30kg (dry weight) and repacked in plastic pots (26 x 36cm and 25cm deep). To test the homogeneity of the radioactivity distribution a sample was taken from each pot and the activity measured by γ-spectroscopy. The coefficient of variation of these activities was 0.285 to 0.308, indicating an acceptable homogenous distribution. On December 13, 2001 one seed of maize (Zea Mays L. cv. Corso) was placed in the middle of each pot at a depth of 3 to 4cm. On the same day, the same amount and type of fertilizers applied in the field were manually applied in solid form onto the soil surface of each pot. Plants in the greenhouse grew under the following conditions: 16h photoperiod, day/night temperatures 25°C/20°C, 40-50% relative humidity of ambient air, and 300 μmol photons m$^{-2}$ s$^{-1}$ minimum light intensity (provided as artificial light by 400W DL/BH Lamps, Eye, Japan). Plants were watered with Osmosis I water by a time-controlled automatic watering device. The total amount of water given to the plants for two months was about 300mm, approximately equivalent to the amount of rainfall (Table 1.3). No leaching occurred in the pot experiment. During the growing period plant height was measured regularly. On February 13, 2001, after two months of growth when plants reached the flowering stage, the stem was cut 1cm above the soil level. Two core samples (20cm long and 5cm diameter) were taken from each pot at 5cm and 13cm from the stem, using a hand-driven auger. Identical procedures as described in the field experiments were used for plant and root analysis.

**Batch experiments**

*Experimental design:* Untreated soil was collected from the A$_h$ horizon of the same field site. Special care was taken to avoid any structural disruption of the soil aggregates during sampling and transportation to the laboratory. The collected soil was air-dried at room temperature for one day and divided into two parts, one part left untouched (un-sieved soil) and one part sieved at 6mm (sieved soil). Portions of 50g (dry weight) of the sieved and un-sieved soil were weighed in 250mL bottles and 150mL of nanopure water was added. Three batch experiments of four replicates of the two soil treatments were conducted and the following properties measured: (a) concentration of water soluble radionuclides; (b) the concentration of water soluble Cs, Co, K, Mn and Zn, and (c) the concentration of water soluble total organic carbon (TOC), inorganic carbon (IC) and the solution pH. Bottles were closed with a plastic lid and were rigorously shaken by hand. A solution (1mL) of the four radionuclides bringing the same concentrations (Bq g$^{-1}$ of soil dry weight) as those used in the field and pot experiments was applied to each sample of the unsieved and sieved soil, respectively, in the first batch experiment (set a). The experiment was carried out at room temperature. Solution was sampled after 30min., 1h, 2h, 3h, 1d, 2d, 3d, 7d and 14d, respectively. Before sampling, all containers were quickly hand-shaken and 1ml of the
Radionuclides uptake by maize grown under field and pot conditions

Suspension was sampled for stable and radioactive Cs, Co, Mn and Zn and for K and 4mL for the measurements of TOC, IC and pH. These samples were filtered through a 0.02 μm filter and pipetted into Eppendorf tubes. The use of an end-over-end shaker was avoided to minimize the disturbance of the aggregates. The concentration of water-extractable Zn, Mn, Co, Cs and K were measured by ICP-MS, radionuclide concentration was measured by γ-spectrometry and TOC and IC were measured by FORMACS® TOC/TN analyzer (Skalar analytical B.V., the Netherlands).

Aggregate stability test: The effect of water on the aggregate stability was studied by comparing dried and wet soil samples for both sieved and un-sieved soil treatments. Wet soil samples were taken from the batch experiment (from the set b described above) when it was completed. Dry soil samples were taken from the left-over sieved and un-sieved soil, which were collected for the batch experiment. Four replicates of 50g (dry weight) were prepared. A modification of the wet-sieving procedure described by Yoder (1936) was used. The pile of sieves was lowered and raised five times manually, instead of using a mechanical arrangement, at intervals of 30 seconds. The entire experiment was performed by a single person. A low standard error was obtained within the four replicates, indicating an acceptable reliability of the method.

γ-Spectrometry
All samples were analyzed for 65Zn (half-life t1/2=243.9d, 1115.55keV), 57Co (t1/2=271.7d, 122.06 keV), 54Mn (t1/2=312.2d, 834.84 keV) and 134Cs (t1/2=2.07y, 475.34 keV) at the γ-spectrometry laboratory of EAWAG (Swiss Federal Institute for Environmental Science and Technology, Dubendorf, Switzerland) using high purity Ge detectors. Plant, root and soil samples were measured with flat crystals. Radionuclide activities were determined in Bq g⁻¹ (dry weight); decay corrected to the common date of July 18, 2001, 12.00h. The measurements errors were 5 to 10%. Geometry correction and calibration are based on standard solutions.

Statistical analysis
Significant differences between the mean values, calculated for plant dry matter production, plant uptake and root weight density, were tested by analysing the variance (ANOVA) and Duncan’s multiple range test. All tests were conducted at the 5% significance level. The analyses were performed with Statgraphics® software version 3.1, (Manugistics Inc., 1997).

Results
Plant growth and radionuclides uptake in the field and in the greenhouse
After two months of growth plant height and shoot dry matter production did not differ significantly between maize grown in the field and in the greenhouse (Table 1.4). The higher shoot biomass observed in the field can be explained by the solar irradiation which was probably higher in the field compared to the
Radionuclides uptake by maize grown under field and pot conditions

Values of root weight density (Table 1.5) obtained in the upper 0-20 cm of the soil profile in the field experiment did not differ from those obtained in the pot experiment, indicating that root development and distribution was similar in both experiments. In the field experiment root weight density values showed an abrupt decrease between 20 cm and 40 cm of soil depth. The high bulk density of the B horizon (Table 1.1) might have impeded root penetration and development and might have caused a reduced transport of radionuclides and dye tracer to the subsoil.

Table 1.4 Plant height and dry matter measured after two months of maize grown in the field and under greenhouse conditions.

<table>
<thead>
<tr>
<th></th>
<th>Field experiment</th>
<th>Pot experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean  SE*</td>
<td>Mean  SE*</td>
</tr>
<tr>
<td>Plant height (cm)</td>
<td>158.5 2.61</td>
<td>147.6 3.71</td>
</tr>
<tr>
<td>Dry matter (g)</td>
<td>54.6 6.42</td>
<td>36.0 3.75</td>
</tr>
</tbody>
</table>

* SE: standard error.

n.s.: not significant

Table 1.5 Root weight density values obtained from the field experiment (at two different depths, 0-20 cm and 20-40 cm) and from the pot experiment.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Field experiment (g cm⁻³)</th>
<th>Pot experiment (g cm⁻³)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5cm*</td>
<td>40cm*</td>
</tr>
<tr>
<td>0-20</td>
<td>0.002 ± 0.001</td>
<td>0.002 ± 0.001</td>
</tr>
<tr>
<td>20-40</td>
<td>0.0007 ± 0.0001</td>
<td>0.0001 ± 9.5x10⁻⁵</td>
</tr>
</tbody>
</table>

* n.m.: not significant

The concentrations of P and N measured in the shoots were higher in the plants grown in the field than in the greenhouse (Table 1.6). Since 2.5 to 3.5 mg P g⁻¹ dry matter are needed in the aerial parts of maize for optimum growth (Bergmann, 1992) the two groups of plants had sufficient levels of P. The determination of critical N concentration (%Nₑ) (Plénet & Lemaire, 1999) gave a value of 19.4 g N kg⁻¹, thus indicating a slight N deficiency in the two groups of plants. In both experiments the concentration of K measured in the shoots was within the range required for a normal growth (17-23 mg K g⁻¹ dry matter; Bergmann, 1992) (Table 1.6).

The recovery of ⁵⁴Mn and ⁶⁵Zn in the aerial parts of maize was similar in the field and in the greenhouse experiment (Table 1.7). Similarly, no significantly different concentrations of Mn and Zn were observed in the aerial parts of the two groups of plants (Table 1.6). The recovery of ⁵⁷Co was two times higher in the shoots of maize grown in the field (Table 1.7), while no differences of total Co concentration were observed between the two groups of plants (Table 1.6).
Table 1.6 Concentration of Mn, Zn, Co, Cs, P, N and K in the shoots of maize after two months of growth under field conditions (field experiment) and in the glasshouse (pot experiment). The significance of the difference between the two experiments is given at $P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. n.s. not significant.

<table>
<thead>
<tr>
<th>Element</th>
<th>Field experiment</th>
<th>Pot experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (g kg$^{-1}$ dry matter)</td>
<td>SE</td>
</tr>
<tr>
<td>Mn</td>
<td>0.02</td>
<td>0.07</td>
</tr>
<tr>
<td>Zn</td>
<td>0.04</td>
<td>0.1</td>
</tr>
<tr>
<td>Co</td>
<td>5x10$^{-4}$</td>
<td>0.003</td>
</tr>
<tr>
<td>Cs</td>
<td>1.3x10$^{-4}$</td>
<td>1x10$^{-4}$</td>
</tr>
<tr>
<td>P</td>
<td>3.6</td>
<td>0.01</td>
</tr>
<tr>
<td>N</td>
<td>16.4</td>
<td>0.06</td>
</tr>
<tr>
<td>K</td>
<td>20.1</td>
<td>0.06</td>
</tr>
</tbody>
</table>

Table 1.7 Recovery of applied radionuclides in the aerial parts of maize. In the field radionuclides were applied onto the soil surface while in the greenhouse the soil has been sieved and homogeneously labelled with the radionuclides before being repacked in pots. The significance of the difference between the two experiments is given at $P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. n.s. not significant.

<table>
<thead>
<tr>
<th>Element</th>
<th>Field experiment</th>
<th>Pot experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean$^\dagger$</td>
<td>SE</td>
</tr>
<tr>
<td>$^{54}$Mn</td>
<td>0.1</td>
<td>0.07</td>
</tr>
<tr>
<td>$^{65}$Zn</td>
<td>5.5</td>
<td>0.4</td>
</tr>
<tr>
<td>$^{57}$Co</td>
<td>0.009</td>
<td>0.0007</td>
</tr>
<tr>
<td>$^{134}$Cs</td>
<td>0.1</td>
<td>0.008</td>
</tr>
</tbody>
</table>

$^\dagger$ [shoot activity/total activity applied per plant [Bq Bq$^{-1}$ x 10$^3$]]

Ten times more $^{134}$Cs was recovered in the shoots of maize grown in the field than in the shoots of maize grown in the greenhouse whereas the total concentration of Cs in shoots was not statistically different. The calculation of the specific activity (SA) for Zn, Mn, Cs and Co showed that the isotopic composition of maize grown under field or greenhouse conditions was similar for Mn and Zn but it was significantly higher in the field than in the greenhouse experiment for Cs and Co (Table 1.8).

Table 1.8 Specific Activity of Mn, Zn, Co and Cs calculated for the aerial parts of maize after two months of growth either under field conditions (field experiment) or in glasshouse (pot experiment). The significance of the difference between the two experiments is given at $P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. n.s. not significant.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Cs</th>
<th>Co</th>
<th>Mn</th>
<th>Zn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable element in shoot (mg)$^\S$</td>
<td>7x10$^{-9}$</td>
<td>2.8x10$^{-10}$</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Radionuclides recovery$^{\dagger\dagger}$</td>
<td>0.1</td>
<td>0.01</td>
<td>0.009</td>
<td>0.004</td>
</tr>
<tr>
<td>Specific Activity$^\dagger$</td>
<td>7.5x10$^7$</td>
<td>3.5x10$^7$</td>
<td>0.33</td>
<td>0.22</td>
</tr>
<tr>
<td>Significance test</td>
<td>**</td>
<td>*</td>
<td>n.s.</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

$^\dagger$ Obtained from values shown in Table 1.6.

$^{\dagger\dagger}$ Obtained from values shown in Table 1.7.

$^\S$ Calculated as: radionuclide recovery/stable element in shoot (mg)
Displacement of surface-applied radionuclides and relation between roots and PFP distribution patterns

Surface applied radionuclides were distributed heterogeneously in the soil profile. All four radionuclides applied showed significantly higher concentration in the preferential flow paths compared to the soil matrix. They were more concentrated in the upper 0-15cm of soil depth and displayed much lower concentration either in the PFP or in the matrix with depth (Figure 1.2).

The analysis of the overlaid root and PFP maps (Figure 1.3) showed that at 20 cm depth PFP covered 11% of the analyzed area and that 15% of the roots were in the PFP. At 40 cm depth, PFP covered 6% of the analyzed area and 9% of roots were in these areas. Hence, the roots showed a moderate preference for the currently active preferential flow paths.

Figure 1.2 Concentration of $^{54}$Mn, $^{65}$Zn, $^{57}$Co and $^{134}$Cs measured in the soil matrix (unstained) and in the preferential flow paths (stained). Mean and standard errors (error bars) are given.
Radionuclides uptake by maize grown under field and pot conditions

Effect of soil sieving on radionuclides availability as assessed in batch experiments

The pH remained around 7.5 during the first three days and then increased to reach a plateau at 8.2 until the end of the experiment (14 days), probably due to the formation of anaerobic conditions. The total organic carbon concentration in the solution increased steadily from 5 to 10 mg C L$^{-1}$ during the first three days and then increased abruptly to reach a value around 30 mg C L$^{-1}$ that was stable until the end of the experiment. The inorganic carbon concentration remained between 5 and 10 mg L$^{-1}$ during the entire experiment. No differences were observed between the two soil treatments for these parameters (pH, TOC, IC) (data not shown).

During the first three days of the experiment the concentration of all radionuclides in the solution decreased rapidly to reach undetectable values until the end of the experiment (14 days). The concentration of $^{54}$Mn, $^{65}$Zn and $^{134}$Cs decreased more rapidly in the presence of the sieved soil, while the concentration of $^{57}$Co decreased more rapidly in the presence of the unsieved soil (Figure 1.4 and 1.5). However these differences were transient and after a few hours similar results were observed in the sieved and unsieved soil. The concentration of stable Cs, Co, Mn and Zn in the solution remained constant during the first three days of the experiment (Figures 1.4 and 1.5). After three days of experiment, the concentration of stable Co and Mn strongly increased in the solution, with higher increases observed in the sieved soil, suggesting the occurrence of reducing conditions (data not shown).

Throughout the whole experiment the concentration of stable Zn remained constant in the sieved and unsieved soil (data not shown), whereas the concentration of Cs steadily decreased in both soil treatments (Figure 1.5). The concentration of K remained constant in the unsieved soil whereas it increased steadily in the sieved soil, reaching at the end of the experiment a significantly higher (2-fold) value than the one measured in the unsieved soil (Figure 1.5).

The aggregate stability test (Figure 1.6) showed that at the end of the batch experiment water has reduced the percentage of the larger aggregates (8 and 4mm) to half the initial value, doubling the percentage of...
the fraction with smaller size (0.25, <0.125mm) in the sieved and in the unsieved soil.

Figure 1.4 Results of the batch experiment carried out on two soil treatments (a) unsieved (simulating the field soil) and (b) sieved (simulating the potted soil). On the left, concentrations of the radionuclides in the solution at application time $t=0$; on the right, concentration of the stable water soluble Mn, Co and Zn as a function of time. Mean and standard errors (error bars) of four replicates are given.
Figure 1.5 Results of the batch experiment carried out on two soil treatments: (a) sieved (simulating the potted soil), and (b) unsieved (simulating the field soil). On the right, concentration of water soluble Cs and activity of $^{134}$Cs remained in solution at application time $t=0$; on the left, concentration of water soluble K, as a function of time. Mean and standard errors (error bars) of four replicates are given. The significance of the difference in water soluble K between the two soil treatments is given at $^*P<0.05$.

Figure 1.6 Aggregate stability test of the two soil treatments: (a) unsieved, and (b) sieved through 6mm. For each soil treatment two types of samples were used: (1) dry soil with 0.20 m$^{-3}$ m$^{-3}$ of water content (black colour), and (2) wet soil, obtained from samples used in the batch experiment in which 50g of soil remained immersed for 14 days in 150 ml of water (grey colour). Mean and standard errors (error bars) of four replicates are given.

Discussion
Our results show that higher amounts of $^{134}$Cs and $^{37}$Co are taken up by maize grown in a field soil where the radionuclides were applied to the soil surface, whereas similar amounts of $^{54}$Mn and $^{65}$Zn are taken up by the plants grown in the field soil and in potted soil. Hence, our initial hypothesis has been validated for
Radionuclides uptake by maize grown under field and pot conditions

$^{134}$Cs and $^{57}$Co uptake but not for $^{54}$Mn and $^{65}$Zn. These results might be explained by the following factors and processes.

The higher concentration of the surface-applied radionuclides observed in the preferential flow paths in comparison to the soil matrix indicates that they infiltrated heterogeneously in the soil profile due to the structure-induced non-uniform water flow, and consequently a fraction of each radioactive element remained sorbed on the soil particles in the vicinity the PFP. This result supports those of Bundt et al. (2001) and Sinaj et al. (2002) who observed a higher radionuclide activity and P concentration sorbed on the solid in the PFP. The amount of added $^{134}$Cs was not much diluted in the small pool of stable Cs (Table 1.1) and the significantly higher specific activity obtained in the plants grown in the field indicated that they had more access to the added $^{134}$Cs in comparison to the plants grown in the pot experiment. The batch experiment showed no differences in terms of $^{134}$Cs and Cs concentration in the solution between the sieved soil and the unsieved soil, indicating that their availability was similar in the two soil treatments. Recent data (Joner et al., 2004) suggest that arbuscular mycorrhizal fungi (AMF) which establish symbiosis with maize roots do not transport significant amounts of Cs to the roots. This suggests that in order to take up Cs from the soil, a plant has to develop roots within a soil volume containing Cs. Altogether these observations suggest that plants grown in the field might have obtained a substantial amount of their $^{134}$Cs from roots growing in the PFP.

The calculation of the specific activity (SA) for Zn, showed that the isotopic composition of maize grown under field or greenhouse conditions was similar. This suggests that whether maize was grown in the field or in the pot it had access to similar chemical forms of Zn. The results of the batch experiment indicated that both forms of Zn were similarly available in the sieved and in the unsieved soil. The similar uptake of $^{65}$Zn and the similar SA of Zn between plants grown in field and greenhouse conditions are not surprising although higher concentrations of $^{65}$Zn were observed in the PFP in the field. It is indeed known that AMF are able to take up and transport significant amounts of Zn to the roots (Bürkert & Robson, 1994). Since the fungal hyphae can transport $^{65}$Zn from distances of up to 10 cm from the roots (Jansa et al., 2003) and, since hyphae having a diameter of a few μm can penetrate and explore very fine pores, it is possible that they explored the entire surface horizon in the field and the entire soil volume in the pot, hence contributing to the $^{65}$Zn uptake by maize.

The results obtained for $^{57}$Co and $^{54}$Mn are more difficult to explain. The calculation of their specific activity (SA) showed that the composition of maize grown in the field or in the greenhouse conditions was similar for Mn but higher for Co. This indicates that maize grown in the field had more access to the added $^{57}$Co, whereas it had access to similar chemical forms of Mn in both experiments. As reported by Marschner and Dell (1994) and Suzuki et al. (2001) both elements are little transported by AMF and therefore they need to be located in the close proximity of the roots to be taken up in significant amounts.
Since the availability of $^{57}$Co and its stable counterpart was similar in the sieved and in the unsieved soil, as indicated in the batch experiment, we suggest that maize grown under field conditions accumulated more $^{57}$Co than plant grown in the greenhouse, because of a higher rate of uptake of this radionuclide from the PFP, where it was highly concentrated. For Mn however no differences were observed in terms of specific activity and in terms of the availability of $^{54}$Mn and Mn between the sieved and the unsieved soil, as indicated in the batch experiment, despite the higher concentration of $^{54}$Mn in the PFP in the field soil. We suggest that the uptake of Mn has been strongly influenced in both experiments by the activities of rhizosphere micro-organisms as the Mn-reducing microbial group of the fluorescent pseudomonas (Posta et al., 1994) and by microbes oxidizing Mn$^{2+}$.

**Conclusion**

Risk assessment models that are usually based on the simplifying approximation that soils are homogeneous; do underestimate the transfer of certain radionuclides from the soil to the plant, as for instance $^{134}$Cs and $^{57}$Co. Our results suggest that among the environmental factors discussed by Ehlken & Kirchner (2002), the non-uniform distribution of roots and the heterogeneous displacement of trace substances can influence root uptake. One may object that this conclusion applies mainly to un-tilled soils or natural and semi-natural ecosystem but, in ploughed soils, depth distribution of radionuclides can also be non-uniform as documented by Meisel et al. (1991).

However, our results are the product of a short-term field experiment carried out in one soil type and with one crop, thus additional experiments on reproducibility of data for a range of soil types and plant species are needed. Further works are also requested to test whether and to what extent plant uptake of radionuclides is influenced by the activity of a small fraction of the root system growing in areas of radionuclides enrichment, as it has been hypothesized in this work for $^{134}$Cs and $^{57}$Co.

**Acknowledgments**

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Time-dependent redistribution of surface-applied radionuclides and their uptake by maize during a growing season
Abstract

In risk assessment models the soil-to-plant transfer of radionuclides is described by a simple parameter - the transfer factor - which does not take into account the time-dependent variations of radionuclides accessibility by roots occurring at the soil-root interface. We studied the redistribution of four surface-applied radionuclides (\(^{54}\)Mn, \(^{65}\)Zn, \(^{57}\)Co. and \(^{134}\)Cs) in the soil profile and their uptake by corn (Zea Mays) grown on an untilled agricultural soil during a growing season. Surface-applied radionuclides were concentrated in the preferential flow paths in comparison to the soil matrix due to structure-induced non-uniform water flow. Only a small fraction of the root system was located within the preferential flow paths. The recovery of \(^{54}\)Mn in the aerial plant parts increased during the plant growing cycle, while the recovery of \(^{65}\)Zn and \(^{57}\)Co did not show any significant difference between plant at pollen shed and maturity, and the recovery of \(^{134}\)Cs decreased with time. The amount and the seasonal distribution of the precipitation promoting the convective vertical displacement of radionuclides, and their infiltration into the soil matrix caused a decrease of their concentration in the preferential flow paths with time. This induced a reduction of plant available radionuclides during the plant’s growth cycle. Nevertheless, unavailable forms of \(^{54}\)Mn might have been mobilized during temporary reducing conditions or through microbial activity.
Introduction

The radiologically most important radionuclides released to the environment are the long-lived γ-emitters isotopes. Their transfer into the human food chain through uptake by plant roots represents a long-term risk. Various studies have shown a decrease of the transfer factor (TF) of long-lived radionuclides with time (Hird et al., 1996; Noordijk et al., 1992; Squire and Middleton, 1996). This time dependence has been attributed to sorption and fixation to clay minerals for $^{137}$Cs and $^{134}$Cs (Hird et al., 1996), to the growth stage of the plants (Bunzl and Kracke, 1989), and to climatic condition (Sandalls and Bennett, 1992). Ehlken and Kirchner (2002) pointed out that the TF should not be used to describe the dynamics of soil-plant transfer processes because by its definition it averages out the time-dependent redistribution of radionuclides within the rooting zone. In a previous study these authors observed differences in TF of $^{137}$Cs between grasses up to a factor of 100 on long-term trends which they explained by the impact of the differing depth distribution of Cs within the rooting zone of the grass plants (Ehlken and Kirchner, 1996).

Recent studies, carried out in agricultural and forest soils, have shown a heterogeneous distribution of surface-applied radionuclides through the soil profile (Bundt et al., 2000; Centofanti et al., Chapter 1 of this dissertation); which was explained by the structure-induced water flow paths, and their sorption onto the surface of preferential flow paths. Centofanti et al. (Chapter 1 of this dissertation) have also suggested that the small fraction of the roots located within and near the preferential flow paths was responsible for the uptake and translocation of $^{134}$Cs and $^{57}$Co. Thus, these results suggest that the time-dependent redistribution of radionuclides within the rooting zone might influence their uptake by roots.

The aim of this study was to assess the time-dependent variations of the uptake of four surface-applied radionuclides ($^{54}$Mn, $^{57}$Co, $^{65}$Zn and $^{134}$Cs) by maize as affected by their distribution within the soil profile. We postulated that plant uptake increases during the plant growth season because the root system develops and explores an increasing fraction of the soil volume, reaching more frequently radionuclide rich niches. Variation of radionuclide uptake during the growing season of maize (April-September, 2002) was studied in an untilled agricultural soil. Plants were harvested at: (i) leaf development stage 3, (ii) pollen shed, and (iii) maturity. At harvest we analyzed plant biomass production, radionuclide content, and root weight density. After plant harvest, radionuclide distribution in the soil profile and the interrelation between roots and flow paths distribution patterns were also analyzed.

Materials and methods

Soil characteristics: The field experiment was conducted on an untilled agricultural soil near Tänikon (E 8° 54’ 22” / N 47° 28’ 53”), Switzerland, at the Eidgenössische Forschungsanstalt für Agrarwirtschaft und Landtechnik (FAT). The soil is a sandy loam Gleyic Cambisol (FAO, 1999). Soil chemical and physical properties are given in Table 2.1.
Table 2.1 Selected chemical and physical properties of the soil at the field site.

<table>
<thead>
<tr>
<th>Soil Depth</th>
<th>Sand † (g kg⁻¹)</th>
<th>Silt † (g kg⁻¹)</th>
<th>Clay † (g m⁻³)</th>
<th>Organic matter † (g kg⁻¹)</th>
<th>Porosity †</th>
<th>pH ‡</th>
<th>CEC ‡‡</th>
<th>Saturated hydraulic conductivity</th>
<th>K †</th>
</tr>
</thead>
<tbody>
<tr>
<td>(cm)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-20</td>
<td>440</td>
<td>361</td>
<td>199</td>
<td>1.1</td>
<td>13.8</td>
<td>0.54</td>
<td>6.2</td>
<td>18.8</td>
<td>771±2</td>
</tr>
<tr>
<td>20-40</td>
<td>402</td>
<td>344</td>
<td>254</td>
<td>1.2</td>
<td>9.6</td>
<td>0.52</td>
<td>6.6</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

† Based on sedigraph analysis, after 6 min of ultrasound treatment in 0.1% Na-hexametaphosphate.
‡ Schweizerische Referenzmethoden der Eidgenössischen landwirtschaftlichen Forschungsanstalten (1999).
§ pH was measured in a suspension of 1 g of dry soil to 2.5 mL deionized water.
‡‡ CEC: cation-exchange capacity (Thomas, 1982).
† estimated by the method of Dirks and Scheffer (1930) according to FAI, RAC, FAW (1996).
n.d. not determined
The field has a slope of around 2%. The experimental site was untilled since more than 10 years and wheat was grown prior to our experiment. A full description of this experimental site can be found in Anken (2004). On the vertical cut of the soil profile we observed many macropores, primarily earthworm burrows which formed a vertically oriented continuous network of pores.

**Experimental design:** The experimental area was 4.9m long and 4.2m wide, subdivided into 3x3 plots (Figure 2.1). On the plot, plants were distributed in rows with a distance of 0.7m between the rows and a distance of 0.15m between plants of the same row. Each plot contained seven rows of eight plants, totalling 56 plants per plot. The radionuclide and dye tracer solutions were applied on three inner areas (0.6m x 0.7m) encompassing three rows and four plants per row (Figure 2.1). Plants were sampled in these inner areas only. The vegetation-free area outside the inner areas was used as buffer rows. On April 21, 2002, four time domain reflectometry (TDR) probes and four tensiometers were installed at two bare soil positions from the plants’ row and at depths of 10, 20, 45, and 60cm and of 15, 30, 45 and 60cm, respectively. One trench was dug directly adjacent to a plant row, and the other was placed at 50cm from the nearest maize’s stalk (Figure 2.1).

![Figure 2.1 Plot design. (A) Overview of the layout in the field experiment. x= plants; grey rectangles= inner areas, traced with radionuclides and dyes; white areas= buffer area to minimize edge effects; black rectangles= instrumented trench close to the maize row (maize soil) and 40cm from the experimental plots (bare soil); (B) Sampling scheme in the traced areas.](image-url)
Application of radionuclide solution: On April 10, 2002, weeds were burnt and a solution of 1% of Glyphosate (Roundup) was applied at the rate of 0.42 ml m\(^{-2}\). On April 28, 2002, a solution containing \(^{54}\)Mn, \(^{65}\)Zn, \(^{57}\)Co and \(^{134}\)Cs, all in chloride form diluted in 1:100 water solution of 0.1M HCl, was applied onto the soil surface of each inner area (Table 2.2). Radionuclides were purchased at Amersham® (Germany). The solution was applied manually, using a watering can with a sprinkling bar fixed at the end of the spout. To avoid surface ponding the solution was applied in 6 slops of equal amounts of ~6.6L within a period of 6h. 40L of solution contained 18.7, 91.3, 18.8 and 29.2 MBq L\(^{-1}\) of \(^{54}\)Mn, \(^{57}\)Co, \(^{65}\)Zn and \(^{134}\)Cs, respectively were applied on each inner area. This amount of water corresponded to a heavy thunderstorm shower. The radionuclides were dissolved in Osmosis I water of an electrical conductivity of 17\(\mu\)S cm\(^{-1}\) at 25°C. To avoid lateral infiltration toward the rows, a similar amount of Osmosis I water was applied at the same rate on the buffer area outside the central plots.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Chemical form</th>
<th>Specific activity (MBq mg(^{-1}))</th>
<th>Total activity applied (kBq)</th>
<th>Activity applied (kBq m(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>(^{54})Mn</td>
<td>(^{54})MnCl(_2)</td>
<td>74</td>
<td>1415</td>
<td>374.4</td>
</tr>
<tr>
<td>(^{65})Zn</td>
<td>(^{65})ZnCl(_2)</td>
<td>13</td>
<td>217</td>
<td>57.4</td>
</tr>
<tr>
<td>(^{57})Co</td>
<td>(^{57})CoCl(_2)</td>
<td>50</td>
<td>2385</td>
<td>631.1</td>
</tr>
<tr>
<td>(^{134})Cs</td>
<td>(^{134})CsCl</td>
<td>37</td>
<td>138</td>
<td>36.6</td>
</tr>
</tbody>
</table>

Plants: On April 30, 2002, furrows were manually prepared and maize (Zea Mays L. cv. Corso) was sown. After sowing, the soil was fertilized with 110 Kg N ha\(^{-1}\) as NH\(_4\)NO\(_3\) in solid form as recommended for maize production in Switzerland and was mechanically spread onto the whole area. The amount of available P and K in this soil was sufficient to allow a proper maize growth (Anken, 2004). The plants were not irrigated to reduce radionuclides irrigation-induced leaching. During the growing period plant height was regularly measured.

A weather station placed 60m from the site, recorded air and soil temperatures, rainfall and solar radiation (Table 2.3). Plants were harvested from the inner areas at three development stages: (a) June 17-19, 2002, at the leaf development stage 3 (LDS3), (b) July 29-31, 2002, at the pollen shed (PS), and (c) September 28-30, 2002, at maturity (M).

At each harvesting time, one row on one subplot was cut (Figure 2.1). The shoots were harvested by cutting the stem 1cm above the soil level, and then washed with tap water, chopped and oven-dried at 105°C for 24h. The whole shoot was used for plant analyses and, at maturity grains were separated from the rest of the plant. The dry weight was determined and the dry material milled to powder. The ground mass was weighted and transferred into calibrated \(\gamma\)-spectrometry containers.
Table 2.3 Monthly meteorological data recorded during the growing period of the maize plants in the field experiment in 2002.

<table>
<thead>
<tr>
<th>Month</th>
<th>Rainfall (mm)</th>
<th>Air temperature (°C)</th>
<th>Air humidity (%)</th>
<th>Duration of solar radiation (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>June</td>
<td>88.9</td>
<td>15.2</td>
<td>70.8</td>
<td>129.5</td>
</tr>
<tr>
<td>July</td>
<td>107.6</td>
<td>18.3</td>
<td>72.5</td>
<td>142.6</td>
</tr>
<tr>
<td>August</td>
<td>124.5</td>
<td>18.0</td>
<td>78.2</td>
<td>189.0</td>
</tr>
<tr>
<td>September</td>
<td>141.6</td>
<td>11.3</td>
<td>80.1</td>
<td>46.4</td>
</tr>
</tbody>
</table>

_Dye tracers application:_ One week before each harvest occasion a fluorescent tracer, Acid Yellow 7 (AY; Fluka Chemie AG, Buchs, Switzerland), was applied onto the soil surface of each inner area. The area was irrigated for 8h a day over a period of 5 days with an Acid Yellow solution (8 g L⁻¹). The cumulated amount of irrigation was 48L of solution per inner area. The solution was applied with a spraying can which was manually operated. The surrounding area was protected with a tarp to prevent the spreading of the solution to adjacent plots. The fluorescence intensity of the dye does not change when illuminated for a few hours and is pH independent in the range from 4 to 9. The background fluorescence of the soil itself does not interfere significantly with the dye emission (Aeby et al., 2001). After cutting the plants at each harvest occasion, 16L of a water solution containing a second tracer, the food stuff Brilliant Blue FCF (C.I. 42090) (5-6 g L⁻¹), was manually applied onto the soil surface of each subplot over a period of 6h. The dye tracer Brilliant Blue FCF (BB) is well suited to visualize the cumulative flow pattern of the infiltrating water because of its low toxicity, high visibility and high mobility (Flury and Flühler, 1995). The dye tracer BB is slightly more mobile than the tracer AY, but both can be used under field conditions using them as observable surrogates compounds that behave similarly to radionuclides (Aeby et al., 2001). Therefore we applied the dye tracer Acid Yellow to obtain detailed imaging of the preferential flow paths areas in the soil profile excavated after harvest, whereas we used Brilliant Blue FCF to sample the preferential flow paths areas (stained) and the soil matrix (unstained).

Mapping root and flow path distribution: On each plot six horizontal surfaces were prepared at consecutive depths of 0.13, 0.18, 0.2, 0.35, 0.38, and 0.4m. They had an area of 0.6m parallel to the row and 0.5m perpendicularly to the row. On these surfaces we mapped the occurrence of roots intersecting the plane of observation using a filt-tip pen onto polythene sheets with a grid of 5cm x 5cm to systematically locate root occurrence (Tardieu and Manichon, 1986). Root maps were scanned and digitized using Scion Image (version beta 4.02) for recording the (x,y)-coordinates of the individual roots. Images of the distribution of the surface-applied fluorescent Acid Yellow 7 were taken on the same area used for the root mapping by using an imaging device consisting of a high-power xenon lamp and a sensitive charge coupled device (CCD) camera. The fluorescence images (1242 by 1152 pixels) were corrected for non-uniform lighting, changing surface roughness, and varying optical properties of the soil.
profile. A detailed description of the device used and of the image processing procedure is described in Aeby et al. (2001). The corners of the grid borders were used as reference points to superimpose the fluorescence images and the digitized root maps. The area of the stained zones (preferential flow paths, PFP) on the fluorescence images and the number of roots occurring within the preferential flow paths was determined using IDL (version 4.01 of Interactive Data Language, Research Systems Inc., Co).

Analysis of radionuclide distribution on soil horizontal profiles: Soil samples were taken with a small spatula from the regions stained by the BB dye, interpreted as the areas of preferential flow paths, and from the unstained soil matrix. Flow path areas were cut with a sharp knife at 1 cm thickness. Areas surrounding the flow paths were sampled at radial distance of 2 cm. Samples of the soil matrix were taken using the same method. All the samples were oven-dried at 60°C for 48h until they reached a stable weight. Then, they were ground and put in plastic containers for γ-spectrometry measurement. The BB concentration of the soil samples was quantified by centrifuging 3g (dry matter basis) of soil with 30mL of nanopure water repeatedly until the water solution appeared clear (no more blue). The sequential water extracts from each sample were pooled together and the concentration of BB was measured with a UV/visible light spectrophotometer at a wavelength of 630nm. The soil matrix was defined as areas containing less than 0.03 mg g⁻¹ of BB (Penfield et al. 2002).

Root weight density: In each plot six cylindrical cores (20 cm long and 5 cm in diameter) were sampled at two depths (0-15 and 15-30 cm) using a hand-hold auger. At the first harvest only the first 0-15 cm were analyzed. To obtain a realistic representation of the root distribution in relation to the plant position, two samples were taken at 5 cm, two at 15 cm, and two at 30 cm from the plant row, on both sides of the plants’ row (Figure 1). Soil was rinsed off from the root samples using the hydropneumatic elutriation system, (Gillson’s Inc.) developed by Smucker et al. (1982). Roots were freeze-dried for 48h. Root dry weight was determined and the root weight density (g cm⁻³) calculated. Measurements of the radionuclide activities in root samples obtained from the field experiment were erroneous and unreliable due to the fine soil particles adhering on the root epidermis.

γ-Spectrometry
All samples were analysed for ⁶⁵Zn (half-life t₁/₂=243.9d, 1115.55 keV), ⁵⁷Co (t₁/₂=271.7d, 122.06 keV), ⁵⁴Mn (t₁/₂=312.2d, 834.84 keV) and ¹³⁴Cs (t₁/₂=2.07y, 475.34 keV) at the γ-spectrometry laboratory of EAWAG (Swiss Federal Institute for Environmental Science and Technology, Dübendorf, Switzerland) using high purity Ge detectors. Plant, root and soil samples were measured with flat crystals. Radionuclide activities were determined in Bq g⁻¹ (dry weight); decay corrected to the common date of July 3, 2002, 12:00h. The measurements errors were 5 to 10%. Geometry correction and calibration are based on standard solutions.
Statistical analysis

Significant differences between the mean values, calculated for plant dry matter production, plant uptake and root weight density, were tested by Duncan’s multiple range test after ANOVA. All tests were conducted at the 5% significance level. The analyses were performed with Statgraphics® software version 3.1 (Manugistics Inc., 1997).

Results

Plant growth and radionuclides uptake

On June 6, 2002, hail damaged about 1 to 2 plants per inner area. The damaged plants were replaced with 10d old seedlings in order to allow their harvest at pollen shed and at maturity. In Table 2.4 plant height and shoot dry matter production are shown. The hail destroyed part of the leaf and in some cases half of the shoot was cut off, therefore the value of the shoot height of plant at LDS3 was extremely low and showed a large standard error (Table 2.4). The large standard error of the shoot dry matter production of plants at pollen shed is due to the lower dry matter production of the replaced plants (Table 2.4).

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Shoot height (cm plant(^{-1}))</th>
<th>Shoot dry matter (g plant(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf development stage 3</td>
<td>5.4 ± 5.2</td>
<td>0.4 ± 0.2</td>
</tr>
<tr>
<td>Pollen shed</td>
<td>90.1 ± 15.9</td>
<td>51.2 ± 41.2</td>
</tr>
<tr>
<td>Maturity</td>
<td>175.6 ± 14.2</td>
<td>152.1 ± 37.1</td>
</tr>
</tbody>
</table>

Value of the root weight density (Table 2.5) showed a slight increase with time, although not statistically significant between pollen shed and maturity, and showed a significant decrease with increasing soil depth, indicating that root growth was homogenous in late development stages but not in space. These results are in agreement with those of Chassot et al. (2001) who found that root length density of maize grown in an untilled soil decreased strongly with increased depth and horizontal distance from the plant row. The recovery of \(^{54}\)Mn in the aerial part of maize increased with time showing significantly higher values at each subsequent harvest (Table 2.6). The recovery of \(^{65}\)Zn was significantly higher in the aerial part of maize at PS in comparison to the plants harvested at LDS 3. Contrarily, the recovery of \(^{65}\)Zn in the aerial part of maize (shoots and grains) at maturity was lower than at PS, although these differences were not statistically significant. The recovery of \(^{57}\)Co in the aerial part of maize increased from the first (LDS3) to the second harvest (PS), while it was similar in shoots harvested at pollen shed and maturity (Table 2.6). \(^{134}\)Cs was not detectable in the aerial part of maize at LDS3 and at maturity. Only \(^{54}\)Mn and \(^{65}\)Zn were detectable in the grains (Table 2.6).
Table 2.5 Root weight densities measured at three development stages of maize. Samples were taken at two depths (0-15cm and 20-40cm) and at three distances from the plant row (5cm, 15cm, and 30cm) (means and standard errors between four replicates).

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>Root weight density (g cm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Depth 0-15cm</td>
</tr>
<tr>
<td></td>
<td>Distance from the plant row (cm)</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Leaf development stage 3</td>
<td>1 x 10(^{-3}) ± 1 x 10(^{-4})</td>
</tr>
<tr>
<td>Pollen shed</td>
<td>4 x 10(^{-3}) ± 2 x 10(^{-3})</td>
</tr>
<tr>
<td>Maturity</td>
<td>9 x 10(^{-3}) ± 1 x 10(^{-4})</td>
</tr>
<tr>
<td>Significance</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

n.d. not detected  
n.s. not significant
Table 2.6 Recovery of surface-applied radionuclides in the aerial parts of maize grown in an untilled agricultural soil. Plants were harvested three times during the growing season corresponding to the three development stages (means and standard errors of twelve replicates are given). At maturity the recovery of the radionuclides was measured in the reproductive organs (Grain).

<table>
<thead>
<tr>
<th>Growth stage</th>
<th>$^{54}$Mn T</th>
<th>$^{65}$Zn T</th>
<th>$^{57}$Co T</th>
<th>$^{134}$Cs T</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf development stage 3</td>
<td>0.006 ± 0.002</td>
<td>0.02 ± 0.003</td>
<td>4.7x10^{-6} ± 1.3x10^{-6}</td>
<td>n.d.</td>
</tr>
<tr>
<td>Pollen shed</td>
<td>0.15 ± 0.02</td>
<td>0.74 ± 0.13</td>
<td>0.011 ± 0.002</td>
<td>0.01 ± 0.03</td>
</tr>
<tr>
<td>Maturity</td>
<td>0.33 ± 0.03</td>
<td>0.45 ± 0.07</td>
<td>0.012 ± 0.0007</td>
<td>n.d.</td>
</tr>
<tr>
<td>Grain</td>
<td>0.004 ± 0.001</td>
<td>0.14 ± 0.03</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

Plant recovery is expressed as: plant activity (Bq)/quantity applied (Bq) x 10^4
n.d.: not detectable

Displacement of surface-applied radionuclides and relation between roots and PFP distribution patterns

Surface applied radionuclides were distributed heterogeneously in the soil profile. All applied radionuclides showed significantly higher concentration in the preferential flow paths compared to the soil matrix and to the radial 2cm fringe outside them (Figure 2.2 and 2.3).

Figure 2.2 Concentration of $^{54}$Mn, $^{65}$Zn, $^{57}$Co and $^{134}$Cs measured in the soil matrix (unstained), preferential flow paths (stained) and in the 2cm fringe just outside the stained flow paths (Unstained). Samples were taken at harvest time when plant reached pollen shed.
The concentrations of the radionuclides in the first 5-10 cm of soil profile measured at pollen shed were in the range of: 0.64 - 0.008 Bq g\(^{-1}\) for \(^{54}\)Mn, 0.11 - 0.008 Bq g\(^{-1}\) for \(^{65}\)Zn, 1.07 - 0.01 Bq g\(^{-1}\) for \(^{57}\)Co and 0.07 - 0.004 Bq g\(^{-1}\) for \(^{134}\)Cs (R. Penfield, personal communication). No data are available for this horizon at maturity. All four radionuclides were measurable down to a depth of 40 cm. The concentration of \(^{54}\)Mn and \(^{57}\)Co in the preferential flow paths (PFP) decreased with time while those of \(^{65}\)Zn and \(^{134}\)Cs could not be detected at the third harvest.

![Graphs showing concentration of radionuclides](image)

**Figure 2.3** Concentration of \(^{54}\)Mn, \(^{65}\)Zn, \(^{57}\)Co and \(^{134}\)Cs measured in the soil matrix (unstained), preferential flow paths (stained) and in the 2 cm fringe just outside the stained flow paths (unstained). Samples were taken at harvest time when plant reached maturity.

The soil samples taken from the matrix and the radial 2 cm fringe outside the preferential flow paths, laid within the conservative threshold of 0.03 mg of dye per g of soil (Figure 2.4 and 2.5). A significant correlation was obtained for \(^{54}\)Mn (\(R^2 = 0.77\) P value=0.0001) and \(^{57}\)Co (\(R^2 = 0.47\) P value=0.001) at the second harvest when plant reached pollen shed, while at the third harvest points were more scattered and most of them were below the 1:1 line (\(R^2 = 0.37\) P value=0.006 for \(^{54}\)Mn and \(R^2 = 0.34\) P value=0.01 for
For $^{65}$Zn and $^{134}$Cs the correlation was not significant ($R^2 = 0.03$, $P$ value = 0.4 for $^{65}$Zn and $R^2 = 0.01$, $P$ value = 0.6 for $^{134}$Cs at pollen shed and $R^2 = 0.02$, $P$ value = 0.5 for $^{65}$Zn and $R^2 = 0.01$, $P$ value = 0.6 for $^{134}$Cs at maturity) since a much lower activity was measured in the preferential flow paths (Figure 2.4 and 2.5).

---

**Figure 2.4** Relation between Brilliant Blue (FCF) and the concentration of radionuclides in the three soil compartments analyzed: (a) preferential flow paths; (b) 2cm fringe just outside the stained flow path areas (radius 2cm); (c) soil matrix; each point represents the average of the soil samples taken on horizontal profiles at 15, 18, 20, 35, 38, and 40cm depth, respectively. Samples were taken at harvest time when plant reached pollen shed.
Figure 2.5 Relation between Brilliant Blue (FCF) and the concentration of radionuclides in the three soil compartments analyzed: (a) preferential flow paths; (b) 2cm fringe just outside the stained flow path areas (radius 2cm²); (c) soil matrix; each point represents the average of the soil samples taken on horizontal profiles at 15, 18, 20, 35, 38, and 40cm depth, respectively. Samples were taken at harvest time when plant reached maturity. The analysis of the overlaid root maps and fluorescent tracer images (Figure 2.6 and 2.7) showed that at the second harvest, when plants reached pollen shed, the area covered by the dye was smaller than at the third harvest, when plants reached maturity. In the upper 0-30cm of soil depth the dye coverage area and the number of roots occurring in the preferential flow paths were larger (Table 2.7). The ratio between percentage of roots occurring in the stained areas and percentage of stained areas was similar at both harvest occasions.
Figure 2.6 Superimposed maps of root occurrence (white circles) and fluorescence images of the distribution and concentration of the surface-applied fluorescent tracer (Acid Yellow). Horizontal profiles were cut at different soil depths (15, 18, 20, 35, 38, and 40 cm). Plants were at the pollen shed.

Figure 2.7 Superimposed maps of root occurrence (white circles) and fluorescence images of the distribution and concentration of the surface-applied fluorescent tracer (Acid Yellow). Horizontal profiles were cut at different soil depths (15, 18, 20, 35, 38, and 40 cm). Plants were at the maturity.
Table 2.7 Analysis of the horizontal root maps and corresponding fluorescent tracer images (Figure 2.6 and 2.7). Horizontal profiles were prepared at six depths and for each depth the surface area occupied by the stained flow paths and the number of root present within the flow paths area are reported.

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>July</th>
<th>October</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PFP(^5) coverage area (%)</td>
<td>Root presence in the PFP (%)</td>
</tr>
<tr>
<td>15</td>
<td>3.8</td>
<td>10.6</td>
</tr>
<tr>
<td>18</td>
<td>4.9</td>
<td>12.8</td>
</tr>
<tr>
<td>20</td>
<td>3.1</td>
<td>11.9</td>
</tr>
<tr>
<td>35</td>
<td>2.6</td>
<td>8.2</td>
</tr>
<tr>
<td>38</td>
<td>0.4</td>
<td>2.9</td>
</tr>
<tr>
<td>40</td>
<td>0.5</td>
<td>0.9</td>
</tr>
</tbody>
</table>

\(^5\) Stained preferential flow paths

Discussion

Our results showed that the recovery of \(^{54}\)Mn in the aerial plant’s part increased with time as plants developed, while the recovery of \(^{57}\)Co and \(^{65}\)Zn stayed constant and that of \(^{134}\)Cs decreased. Hence, our hypothesis has been validated only for \(^{54}\)Mn. These results might be explained by the following factors and processes.

A higher concentration of the surface-applied radionuclides was observed in the PFP compared to the soil matrix because they have been heterogeneously displaced in the soil profile due to the structure-induced non-uniform water flow. This result supports those reported by Centofanti et al. (Chapter 1 of this dissertation) who, in another field experiment carried out in an untilled agricultural soil, observed a higher concentration of the same surface-applied radionuclides in the PFP relative to those of the soil matrix. In contrast to the mentioned study, in this experiment surface-applied radionuclides reached a depth of 40 cm due to the higher saturated hydraulic conductivity of this soil and to the presence of numerous earthworm burrows. The lack of tillage favours the development of surface-feeding earthworm \(L. \ terrestris\) and allows the large vertical earthworm burrows to persist, potentially becoming important pathways for a rapid infiltration (Edwards et al., 1990).

The concentration of radionuclides in the PFP decreased with time, being lower in the last harvest in comparison to the previous one. This can be caused by the distribution of the precipitations during the experiment. Several short-time (1-2 d) rainfall events of relative high intensities occurred during the six months of the experiment (Figure 2.8) and caused fluctuation in the soil water content (Figure 2.9).
Figure 2.8 Diagram of the rainfall intensity measured during the plant growing season.

Thus, starting from beginning of April until the end of the experiment, periods of 10-15 d showing volumetric water contents of 0.32 m$^3$ m$^{-3}$ alternated with periods of 20-30d of water contents close to saturation (0.40 m$^3$ m$^{-3}$). These fluctuations were predominant in the upper 0-15cm of the soil profile and partly in the 15-30cm soil depth, where a higher proportion of roots and of preferential flow paths was observed. It is very unlikely that the fluctuations in soil water content at these soil depths were caused by root water uptake, since a similar and more pronounced pattern of water content fluctuation was observed in the nearby bare soil (Figure 2.9). These results strongly suggest that rapid drainage most probably occurred through the macroporous preferential flow pathways. An increase in the PFP surface area was observed at the last harvest occasion, indicating an expansion of the water flow paths. Thus, since radionuclides were sorbed on the soil surface surrounding these pathways and none or very small concentrations were found in the surrounding soil, they might have been transported further down in the soil profile by the flowing water. Stamm et al. (1998) indicated that even strongly sorbing solutes such as pesticides can reach deep soil horizons by preferential flow, because the flow velocities in the PFP are high and the ratio of sorptive surfaces to solution is small. An additional factor which might have caused the leaching of some fractions of the radionuclides might have been the application of the fluorescent dye tracer solution which infiltrated the surface horizon at a high rate one week before harvesting.
The pattern of spatial distribution of the surface-applied radionuclides had an important effect on their uptake by the plants. Firstly, it appears that roots growing in the PFP - though only a small fraction of the root system - must have been responsible for the uptake of the radionuclides, because they were concentrated in the PFP and only small amounts of them were found in the soil matrix. Similar conclusions were reached in a previous study for $^{137}$Cs and for $^{57}$Co (Centofanti et al., Chapter 1 of this dissertation). Secondly, the redistribution of surface-applied radionuclides through the soil profile during plant growth seemed to influence their uptake by roots. It has been reported by various authors (Absalom et al., 1999; Ehlken and Kirchner, 1996; Krouglov et al., 1997; Rigol et al., 2002; Sanzharove et al., 1994) that the transfer of radiocaesium appears to decrease with increasing time between soil contamination and harvest of crops. This is attributed to a variety of processes including fixation to soil minerals, incorporation by soil micro-organism and climatic conditions (Ehlken and Kirchner, 1996; Krouglov et
al., 1997). However, in our experiment no uptake of radiocaesium occurred at maturity because it was transported down into and through the rooting zone. It is known that Cs is strongly retained in micaceous clay minerals (illite), and thus Cs migration rates are generally less than 2 cm y\(^{-1}\) (Delvaux et al., 2001). Such migration rates very likely only apply to cases where matrix flow dominates, that is in absence of preferential flow. It is however possible that a fraction of Cs migrated with fine-dispersed suspended micaceous particles, the transport and distribution of which is controlled by the hydrological properties of the soil (Kuznetsov et al., 2001). Furthermore, the high soil K content in the upper 0-20cm of soil profile might have caused a lower Cs fixation to the clay particles thus increasing the Cs mobility within the soil profile. The decrease in the recovery of \(^{134}\)Cs in the aerial plant parts between plants at pollen shed and at maturity can be also due to the recirculation of the absorbed \(^{134}\)Cs from the shoot and leaf to the roots (Buysse et al., 1995). Recently, Feller et al. (2000) have shown that in steam-girdling experiments \(^{134}\)Cs was eliminated from the xylem sap of detached wheat shoots and loaded into the phloem during acropetal transport.

The time-dependent variations of \(^{54}\)Mn, \(^{57}\)Co and \(^{65}\)Zn recovery are probably due to the occurrence of periodically occurring reducing conditions which affect their solubility. In the reducing environment of flooded soils Mn, Co and Zn can be desorbed from the oxides and hydroxides minerals and become plant available (Mcbride, 1989). In our experiment the occurrence of periods in which the water content reached saturation might have caused at least temporarily reducing condition which increased the \(^{54}\)Mn, \(^{57}\)Co and \(^{65}\)Zn availability. Thus, even if the ions migrated down the soil profile with time, their recovery did not decrease during the growing season because they might have been available to the root growing in the PFP.

In the case of \(^{65}\)Zn, though most of it migrated out of the rooting zone at maturity, the uptake was not significantly lower than at pollen shed and a high amount was translocated to the grains. It is known that fungal hyphae of AMF can transport \(^{65}\)Zn from distances up to 10cm from the roots (Jansa et al., 2003) and, having a diameter of a few µm, can penetrate and explore very fine pores. Thus, it is possible that they were able to take up trace amounts of \(^{65}\)Zn present in the soil which were not measurable (below the instruments’ detection limit).

In addition, many members of the bacterial and fungal genera Bacillus, Pseudomonas, Arthrobacter, Streptomyces, and Aspergillus can mediate Mn reduction via abiotic reactions with their metabolic end products and/or via enzymatic reaction using Mn\(^{4+}\) as a terminal electron acceptor (Posta et al., 1994). Hence, it is possible that these processes caused an increase of \(^{54}\)Mn recovery in the aerial plant parts with time.
Conclusions

Our experiment has shown that the redistribution of surface-applied radionuclides within the rooting zone can play an important role on time-dependent variations of the soil-to-plant transfer of radionuclides. This effect can, however, vary depending on the type of radionuclide, the amount and distribution of rainfall, and the chemical, physical and microbiological soil characteristics.

The results obtained in our experiment are conditional on the site specific properties and crop. The above analysis applies to a single growth cycle which is the most important phase after a possible fallout event. Analysis of stable isotopes and of macronutrients will be carried out to assess their uptake and to calculate the specific activity.

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Uptake and translocation of $^{134}$Cs by a fraction of the root system of maize as affected by K supply
Abstract
Structure-induced non-uniform water flow induces a heterogeneous distribution of surface-applied radionuclides in the soil profile. This study was conducted to assess the amount of $^{134}$Cs which can be taken up by a single root growing in an area enriched in $^{134}$Cs and that can be translocated to shoots and leaves relative to the total amount of $^{134}$Cs that can be taken up by the whole root system and translocated to the aerial parts of the plant growing in an area homogeneously contaminated with $^{134}$Cs. A split-root experiment was used to simulate the heterogeneous distribution of $^{134}$Cs and roots. Seedlings of maize ($Zea mays$ L. cv Corso) were grown for 14 days in solution culture and then transferred to a two-compartment pot system, where a single root was grown in a $^{134}$Cs contaminated compartment while the rest of the root system was grown in an uncontaminated compartment. Plants with the whole root system growing in a solution contaminated with $^{124}$Cs were used as control. We tested the effect of the competition between Cs and K on the uptake and translocation of $^{134}$Cs by using two K concentrations, 0.2 and 1.05mM. At K concentration of the nutrient solution of 0.2mM the single root was able to take up and translocate to the shoot about 46% of the $^{134}$Cs taken up by the entire root system. This effect was about 2 times lower at 1.05mM K concentration. The translocation of $^{134}$Cs from the root to the shoots did not depend on the external K concentration in the nutrient solution, but it was lower in the split root treatment at both K concentrations.
Introduction

Soils are characterized by a heterogeneous structure caused by the presence of macropores, earthworm burrows, cracks, and pores left by decaying roots which can affect both the displacement of surface-applied nutrients and contaminants and the distribution and growth of new plant roots. It has been shown that, due to structure-induced non-uniform water flow in soils, highly sorbing solutes such as selected radionuclides (Albrecht et al., 2002; Bundt et al., 2000) and pesticides (Flury, 1996) distribute heterogeneously in the soil profile, accumulating within and around macroporous preferential flow pathways. Stewart et al. (1999) showed that, in uncultivated and coarsely structured black vertisols, up to 80% of the root system was located within macropores or closely associated with them. Centofanti et al. (Chapter 1 and 2 of this dissertation) showed that between 10 and 15% of the roots of maize grown in untilled soils were located in the preferential flow paths (PFP). Pierret et al. (1999) and Pankhurst et al. (2002) demonstrated the existence of an environment around soil macropores that is chemically and microbiologically different from the bulk soil, supporting active microbial populations that differ quantitatively and functionally from those present in the bulk soil. Bundt et al. (2000) also observed a significantly higher root biomass of various tree species in the preferential flow paths than in the matrix of an acid brown forest soil.

Centofanti et al. (Chapter 1 of this dissertation) analyzed the influence of non-uniform root densities and of the heterogeneous distribution of radionuclides in the soil profile on plant uptake. They showed that the amount of $^{134}$Cs recovered in the aerial part of maize grown in an untilled agricultural soil, where surface-applied radionuclides accumulated within and near the structure-induced water flow paths, was 10 times higher than the amount of $^{134}$Cs recovered in the aerial part of maize grown in a glasshouse under controlled conditions in the same soil that had been sieved and homogeneously labelled before being repacked in pots. A significantly higher recovery of $^{57}$Co (2-fold) was also observed in the plants grown in the field soil, whereas no differences in the recovery of $^{54}$Mn and $^{65}$Zn was detected between the two experiments. The authors suggested that plants grown in the field conditions might have obtained a substantial amount of their $^{134}$Cs and $^{57}$Co from roots that were growing within and around $^{134}$Cs and $^{57}$Co enriched areas, although only 10 to 15% of the roots were located within these areas of radionuclide enrichment.

The response of roots to the heterogeneous or patchy distribution of nutrients (as N and P) has been analyzed in many studies, some of which have employed the split-root system technique in solution (Drew et al., 1984) while others have used banded fertilization as a temporary concentrated source (Caldwell et al., 1991; Hodge, 2003). Some of these studied have demonstrated that plant roots respond to these nutrient patches displaying morphological (flexibility in architectural patterns) and/or physiological plasticity (altered nutrient uptake capacity and increased ion affinity) (Hodge, 2004). Robinson et al.
Uptake and translocation of $^{134}$Cs by a fraction of the root system (1991) in an experiment with wheat plants harvested at six steps from 13 to 97 d after germination estimated that the mean fraction of the root system likely to have been involved in nitrate uptake were 11 and 3.5% of the total root length in a treatment without N and in a treatment with 200 kg N ha$^{-1}$, respectively. However, it is not known whether a similar response of a small fraction of roots to the patchy supply of nutrient can also be observed in the uptake of locally distributed trace elements such as $^{134}$Cs. Although there is no known role of Cs in plant nutrition (Marschner, 1995), Cs can be transferred into plants due to its physiological similarity with K (Shaw and Bell, 1991). Recent studies have shown that, at low K concentration in the soil solution, its uptake is driven by a high-affinity K transporter that shows little discrimination against Cs, whereas at high external K concentrations (typically above 0.5-1mM) its uptake is driven by a low-affinity system (channel mediated transporter) through which little Cs is transported (Sacchi et al., 1997; Zhu et al., 2000).

We postulate that the uptake of $^{134}$Cs by a single root growing in a $^{134}$Cs enriched area accounts for most of the uptake relative to that of the whole root system growing in an homogeneously contaminated medium and that $^{134}$Cs uptake and translocation is higher when the K concentration of the nutrient solution is low, because of the higher Cs uptake through the high-affinity transport system for K.

The aim of this study was to assess to what extent a small fraction of the root system, growing in areas of $^{134}$Cs enrichment, may contribute to the total uptake and translocation of $^{134}$Cs in relation to its uptake and translocation by the whole root system growing in an homogeneously contaminated medium, and whether this process is strongly affected by the external K concentration. A split-root experiment, carried out in hydroponic system, was used to simulate the heterogeneous distribution of $^{134}$Cs and root. A single root was allowed to grow in a $^{134}$Cs contaminated compartment, while the rest of the root system developed in an uncontaminated compartment. Plants with the whole root system growing in a nutrient solution contaminated with $^{134}$Cs were used as control. We tested the effect of the competition between Cs and K on uptake of $^{134}$Cs, by using two K concentrations: 0.2mM and 1.05mM.

Materials & Methods

Hydroponic system

Maize (Zea mays cv. Corso) seeds of 0.28-0.30 g were selected, put in a substrate composed of 2/3 soil, which was obtained from a previous field experiment (Chapter 1 of this dissertation), and 1/3 sand (0.7mm), and incubated at 28°C for 3 days. Germinated seeds were transferred in a climate chamber for 2 days under the following conditions: a day/night cycle of 16h with 25/20°C, 70% relative humidity, and a light intensity of 300-400μmol s$^{-1}$ m$^{-2}$. Seedlings, homogenous in growth, were chosen to be transferred to 10L plastic (PVC) barrels (25cm×12cm and 22cm deep) containing a continuously aerated nutrient solution. The chemical composition of the nutrient solution is listed in Table 3.1. Two K concentrations
were tested: (a) the K1 treatment at 0.2mM; and (b) the K2 treatment at 1.05mM. The seedlings were placed into 2cm diameter holes drilled into 22 x 12cm plastic plates which were put on the top of the barrels. Through the holes roots gained access to the nutrient solution. The individual plants were vertically sustained by supporting their collar with cotton wool. Four plants were arranged in the holes of each barrel. The nutrient solution was constantly aerated. The water level and pH (6.0-6.5) were kept constant throughout the growing period by changing the solution every 2 days. Plants grew in the greenhouse under the following conditions: 16h photoperiod, day/night temperatures 27°C/20°C, 40-50% relative humidity of ambient air, and 300 μmol photons m⁻² s⁻¹ minimum light intensity (provided as artificial light by 400W DL/BH Lamps, Eye, Japan).

Table 3.1. Composition of the nutrient solution differing only in the K concentration.

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration in nutrient solution (mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K1 treatment (0.2mM)</td>
</tr>
<tr>
<td>Ca(NO₃)₂</td>
<td>5</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>0.15</td>
</tr>
<tr>
<td>KNO₃</td>
<td>-</td>
</tr>
<tr>
<td>CaHPO₄</td>
<td>0.2</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>2</td>
</tr>
<tr>
<td>Fe-EDTA</td>
<td>0.1</td>
</tr>
<tr>
<td>KCl</td>
<td>0.05</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>0.025</td>
</tr>
<tr>
<td>MnSO₄</td>
<td>0.002</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>0.002</td>
</tr>
<tr>
<td>CuSO₄</td>
<td>0.0005</td>
</tr>
<tr>
<td>Na₂MoO₄</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

Split-root experiment

Plants were grown in hydroponic culture as described above. On the 15th day of growth half of the plants were transferred to two-compartment barrels while the other half of the plants were left in the original one-compartment barrels. The two-compartment barrels were made out of 10L barrels divided into two equal compartments with a plastic (PVC) panel fixed in the middle of the barrel’s short side. Silicone gel was used to fix the vertical panel and to create an impermeable barrier between the two compartments. Both compartments were filled with either the K1 or the K2 nutrient solution. On the same day, a target ¹³⁴Cs activity of 9.2 kBq (in chloride form and diluted in a 1:100 water solution of 0.1M HCl, with a specific activity of 37MBq mg⁻¹, purchased at Amersham®) was added to one of the two compartments (referred to as contaminated compartment) and to the single-compartment barrels (referred to as control). One seminal root of each seedling was placed in the contaminated compartment and the rest of the root system was placed in the uncontaminated compartment, whereas the whole root system was placed in the single-compartment barrels (Figure 3.1).
Twelve barrels per K treatment were prepared, with a total of 48 seedlings and two seedlings per barrel, for the control and the split-root experiment (Figure 3.2). Growing conditions in the greenhouse were as described above. The experiment lasted for five days during which the nutrient solution was not changed but the water level was kept constant by adding deionised water (from 25 to 30 mL day$^{-1}$ compartment$^{-1}$).

![Split-Root Experiment](image1)

![Control Experiment](image2)

**Figure 1.** Schematic design of the experimental set-up for the split-root experiment and of the control experiment. The grey and white colours of the container for the nutrient solution and roots indicates the contaminated and uncontaminated compartments.

**Plant harvest**

Plants were harvested at 12h, 24h, 48h, 72h, 96h and 120h after the addition of $^{134}$Cs in the nutrient solution. At each harvest 16 plants were harvested: 4 of the control experiment and 4 of split-root experiment for the K1 and K2 treatments, respectively (Figure 3.2). Shoots were cut with a sharp knife 0.5 cm above the plastic panel covering the barrels. Shoots, roots and the single root from the split-root experiment were kept separated. Root samples were first washed with a solution of 10 mL HCl (0.1 M) diluted in 100 mL of water over a period of 2 min. to remove the surface film of radio-labelled solution and then were blotted dry on a paper towel. All samples were weighed, oven-dried at 60°C for 3 days, weighed again and milled to powder. Part of each sample was put in plastic tubes calibrated for the $\gamma$-spectrometry measurements, while the rest was used for measurements of K concentration. After harvest, aliquots (5 and 1 mL) of the nutrient solution were sampled from each barrel of the control experiment and from both compartments of the split-root experiment. The 5 mL samples were used to measure the K concentration in the nutrient solution, while the 1 mL samples were used for the $^{134}$Cs analysis.
Harvest at: $t_1$, $t_2$, $t_3$, $t_4$, $t_5$, $t_6$

Set up of the control experiment for K1 and K2

Set up of the Split-root experiment for K1 and K2

**Figure 3.2.** Design of the set up of the control and split-root experiment. Rectangles=barrels; X=plants; $t_1$, $t_2$, $t_3$, $t_4$, $t_5$, $t_6=12h$, 24h, 48h, 72h, 96h, and 120h after the injection of $^{134}$Cs in the nutrient solution. Four plants harvested at each time and for each experiment are considered replicates.

$^{134}$Cs measurement

The concentration of $^{134}$Cs (half-life $t_{1/2}=2.07y$, 475.34 keV) was measured at the $\gamma$-spectrometry laboratory of EAWAG (Swiss Federal Institute for Environmental Science and Technology, Dübendorf, Switzerland) on high purity Ge detectors. Plant, roots, and nutrient solution were measured with flat crystals. Radionuclide activities were determined in Bq g$^{-1}$ (dry weight) for solid samples and in Bq mL$^{-1}$ for the solution samples. The activities were decay corrected to the common date of May 14, 2003, 12:00h. Measurements error was 5 to 10%. Geometry correction and calibration are based on standard solutions.

$K$ measurements

Ground samples (0.25g) of shoots, roots, and seeds were weighed in plastic tubes and 2mL of nanopure water was added. To obtain a complete wetting of the dry material samples were put in an ultrasonic bath (Bandelin, SONOREX Super, RK 510, Switzerland) for 10s and then 4mL of HNO$_3$ (65%, Suprapure, Merck) and 2ml of H$_2$O$_2$ were added. After 30 min. the samples were digested in the microwave oven (MLS mega 1200, Germany) using the following program: 5min., at 269W and 180°C, 8 min. at 600W.
and 185 °C, and 7 min. at 350W and 180°C. After digestion, samples were diluted to 50ml with nanopure water and filtered under vacuum through a 0.45μm Millipore membrane. K concentration of the seed, plant, root, and nutrient solution was measured by ICP-MS (Agilent, 7500 C). The concentration of K in the seeds was about 3.5 mg g⁻¹ with a standard variation of 0.8. This value was withdrawn from the K uptake values measured in the shoot and roots.

**Calculation of shoot: root ratio**

The shoot: root ratio of ¹³⁴Cs was calculated as: Bq (g shoot⁻¹) / Bq (g root⁻¹), and the shoot: root ratio of K as: mg (g shoot⁻¹) / mg (g root⁻¹), as proposed by Staunton et al. (2003).

**Statistical analysis**

Statistical analysis was carried out with Statgraphics® version 3.1 (Manugistics, 1997). Mean values for plant dry matter production, root parameters and ¹³⁴Cs and K uptake between the control and the split-root experiment at each K treatment were compared using paired Student t test. The effect of the K treatments on ¹³⁴Cs uptake was analyzed by one-way ANOVA and Duncan’s multiple range test. All tests were conducted at the 5% significance level.

**Results**

**Dry matter production**

The shoots of the control and of the split-root experiment are referred to as control shoot and split shoot, respectively, whereas root of the control and of the split-root experiment are referred to as control root and split-root, respectively; the one root grown in the contaminated compartment of the split-root experiment is referred to as single root (Figure 3.1).

None of the differences in dry matter production between control and split shoot and control and split root (single root + split root) for the two K treatments were statistically significant (Figure 3.3). Similarly, no significant differences were observed between the K1 and K2 treatment. This indicates that the K concentration of the nutrient solution in the K1 treatment was not limiting for plant growth and that the extra source of nitrogen added in the K2 treatment had no effect on plant growth. At the end of the experiment the dry matter of the single root represented 21% and 15% of the dry matter of the control root grown in the K1 and K2 treatment, respectively.
The variations in $^{134}\text{Cs}$ uptake during the experiment (Figure 3.4) are due to the variations in dry matter production of the plants at each harvest occasion. Uptake of $^{134}\text{Cs}$ was significantly higher in the control plants and only the differences between the control shoot and the split shoot in the K1 treatment were not statistically significant. Significantly higher uptake values were observed in the K1 treatment in comparison to K2 treatment for both control and split-root experiment.
Figure 3.4. Dynamic of $^{134}$Cs uptake measured at the end of each harvest. Vertical bars indicate standard errors calculated from four replicates.

The higher uptake of $^{134}$Cs by plants grown in the K1 treatment is reflected by the differences in the concentration of $^{134}$Cs in the nutrient solution of the two K treatments (Figure 3.5). In the split-root experiment at the last harvest and in both K treatments a small concentration of $^{134}$Cs was measured in the roots grown in the uncontaminated compartment (Figure 3.3) and the $^{134}$Cs concentration was higher in the root grown in the K1 treatment than in the K2 treatment.

In the K1 treatment the uptake of $^{134}$Cs by the whole plant of the control experiment and by the whole plant of the split-root experiment represented 38% and 21% of the $^{134}$Cs applied, respectively. Six days after adding $^{134}$Cs to the solution the single root took up 47% of the $^{134}$Cs taken up by the control root. The quantity in the shoot was 46% relative to the control shoot. In the K2 treatment, the uptake of $^{134}$Cs by the whole plant of the control experiment and by the whole plant of the split-root experiment was 2% and
0.3% of the $^{134}$Cs applied, respectively. The single root took up 10% of the amount of $^{134}$Cs taken up by the control root and the quantity in the shoot was equal to 20% in respect to the control shoot.

![Figure 3.5.](image)

**Figure 3.5.** Concentration of $^{134}$Cs in the nutrient solution measured at the end of each harvest. Vertical bars indicate standard error calculated from four replicates.

**K uptake**

The variations in K uptake during the experiment were due to the variation in the biomass production of the analyzed plant parts (Figure 3.6). K uptake by plants grown in the K1 treatment was not statistically different from that by plants grown in the K2 treatment, but at the end of the experiment the K concentration in the K1 treatment was depleted in all three compartments (Figure 3.7). Similarly, no statistically significant differences were observed between the control and the split-root experiment.
Figure 3.6. Dynamic of K uptake measured at the end of each harvest. Vertical bars indicate standard error calculated from four replicates.

Figure 3.7. Concentration of K in the nutrient solution measured at the end of each harvest. Vertical bars indicate standard error calculated from four replicates.
**Shoot: root transfer of $^{134}$Cs and K**

The shoot: root concentration ratio of $^{134}$Cs and K did not depend on the K concentration in the solution. In both, the control and split-root experiments, the translocation of $^{134}$Cs to the shoot started within the first 12 h after adding $^{134}$Cs into the solution was faster in the control experiment (Figure 3.8).

![Figure 3.8](image)

**Figure 3.8.** Ratio of concentration of $^{134}$Cs measured in shoot and root. Vertical bars indicate standard error calculated from four replicates.

The shoot: root ratio of K showed no significant differences between the two experiments and no variation with time (Figure 3.9). For both $^{134}$Cs and K the concentration ratio between shoot and root was significantly higher in the control experiment than in the split-root experiment. In addition, for $^{134}$Cs the concentration ratio showed a significantly increasing trend with time in both experiments and in both K treatments ($R^2=0.75$ and $P$ value$=0.0002$ for the control experiment and $R^2=0.71$ $P$ value$=0.0006$ for the split-root experiment).

![Figure 3.9](image)

**Figure 3.9.** Ratio of concentration of K measured in shoot and root. Vertical bars indicate standard error calculated from four replicates.
Discussion
The results of the split-root experiment confirmed our initial hypothesis and indicated that a single root growing in a compartment enriched with $^{134}$Cs in the presence of low K concentrations is able to take up a significant amount of $^{134}$Cs relative to its uptake by the whole root system. The contribution of the single root relative to the total $^{134}$Cs uptake was five times higher at low (0.2mM) K concentration in the nutrient solution than at K concentrations higher then 1mM. Our results confirmed that, although Cs plays no role in the nutrition of plants, under low external K concentration Cs is taken up in high amounts due to plant K demand and the physiological similarity to K. In the split-root experiment plants grown at lower external K concentration depleted the solution from its K after 5 days causing a higher $^{134}$Cs uptake compared to the plants grown at higher external K concentration. In our experiment, in both the K1 and K2 treatments, plants of the control and of the split-root experiment continued to translocate increasing amounts of $^{134}$Cs to their shoots with time. Our results indicate that within 6 days after adding $^{134}$Cs into solution the distribution of $^{134}$Cs from the root to the shoot did not attain equilibrium. This increasing trend was not observed for K. This difference can be due to the experimental conditions. In the case of K, plants were given the same pre-treatment and thus were brought to equilibrium with the external solution, whereas to simulate accidental release of $^{134}$Cs, $^{134}$Cs was added as a pulse without pre-treating the plants. The higher shoot: root ratio of $^{134}$Cs in the control experiment then in the split-root experiment can be explained by the translocation of small amounts of $^{134}$Cs to the rest of the root system grown in the uncontaminated compartment. Recently, Feller et al. (2000) have shown that in steam-girdling experiments where the transfer of $^{134}$Cs with the xylem sap of detached wheat shoots was stopped $^{134}$Cs was loaded into the phloem during acropetal transport. This suggests that in our experiment the adsorbed $^{134}$Cs was translocated to the stem and leaf and it was then redistributed to the other part of the root system via phloem transport.

In contrast to the uptake of $^{134}$Cs by the roots, the $^{134}$Cs in the shoot: $^{134}$Cs in the root ratio did not depend on the external K concentration. This observation is in agreement with the data of Staunton et al. (2003) who found no significant effect of K supply on the $^{137}$Cs in the shoot: $^{137}$Cs in the root ratio of various plant species. Contrarily, Buysse et al. (1996) found that at K solution concentration of 0.25mM the $^{137}$Cs concentration ratio between shoot and root of sunflower was lower than that at higher (>1.0mM) K concentration, indicating an increase of the proportion of Cs retained by the roots at low external K concentration. They found no effect of external K concentration on the concentration of K in the shoots. However, in this study increases in K concentrations were compensated for by concomitant decreases in the concentration of Ca- and Mg-salts in the nutrient solution, which might have caused impairments in the uptake transports system.
In our experiment similarly to $^{134}$Cs, the quantity of K transported to the shoots was similar in the two K treatments applied but the shoot: root ratio of K was higher than that of $^{134}$Cs. This suggests that the transport and redistribution processes of K within the plant selectively discriminates against Cs similarly to the active high-affinity K transport system. Recently, Gaymard et al. (1998) have shown that outward rectifying K channels (SKOR) expressed in the root stele (pericycle and stellar parenchyma) are able to passively secrete K into the xylem sap, contributing to about 50% of K translocation toward the shoots of Arabidopsis. However, Ache et al. (2001) have recently identified a K channel (VfK1) expressed in both source and sink phloem tissue of *Vicia faba* which is suggested as being involved in phloem sap K loading in sources and unloading in sinks (Lacombe et al, 2000). The expression analysis of this channel indicated sensitivity to light, photo-assimilates, and hormones suggesting that it might play a role in controlling phloem K transport in relation to sugar production and translocation (Very and Sentenac, 2003).

**Conclusions**

Our results have shown that a single root has the capacity of taking up high proportions of $^{134}$Cs when grown in $^{134}$Cs enriched areas relative to the uptake by the whole root system. Under low external K concentrations significant amounts of $^{134}$Cs are highly taken up by the roots. An increasing fraction of the absorbed $^{134}$Cs is continuously translocated to the shoots, independently of the external K concentration. One might object that the results obtained in the experiment at low K level do not represent realistic soil conditions because of the limited K supply. However, Smolders et al. (1996) have shown that taking into account of actual rhizosphere K concentration, reflecting the root-induced depletion of K, allowed a better prediction of the measured $^{134}$Cs uptake then when considering the concentration of K in the bulk soil. It appears that the processes occurring at the interface between the areas of $^{134}$Cs enrichment and the roots located within and near these areas might have relevant effects on the soil-to-plant transfer of $^{134}$Cs. These results obtained in a controlled experiment with young plants grown under greenhouse conditions provide a basis for explaining the data obtained by Centofanti et al., 2005 under the complex field conditions.

**Acknowledgments**

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Modelling $^{134}$Cs uptake by maize using the Barber-Claassen approach
Abstract

One of the major pathways for the entry of radiocaesium into the human food-chain is through its transfer from the soil to the plant, it is therefore important to make long-term prediction of its fate in the soil system in order to limit its entry into the food-chain. This paper describes the application of the mechanistic model developed by Barber and Claassen for predicting transfer of radiocaesium (\(^{134}\text{Cs}\)) to the aerial plant’s part. The experiment was performed on maize (\textit{Zea Mays} L. cv. \textit{Corso}) grown in a Gleyic Cambisol and at two plant development stages: leaf development stage 3 (LDS3) and pollen shed. Predicted value of \(^{134}\text{Cs}\) recovery in the aerial plant’s part at pollen shed was one order of magnitude higher than that measured, while predicted values of K recovery was two-times higher than that measured. It was not possible to measure the Michaelis-Menten parameters for \(^{134}\text{Cs}\) and K with plant at LDS3. Hence due to the fact that only one uptake value - that of plant at pollen shed – was obtained a regression analysis between measured and predicted value could not be performed. Various factors could have influenced the overestimation of \(^{134}\text{Cs}\) uptake by the model, including the importance of competition between Cs and K which occur both at the soil and root level.
Introduction

Two radioisotopes of Cs (\(^{134}\text{Cs} \ t_{1/2}=2.06\text{year}\) and \(^{137}\text{Cs} \ t_{1/2}=30.2\text{year}\)) are of environmental concern due to their relatively long half-lives, emission of \(\gamma\) and \(\beta\) radiation during decay, and rapid incorporation into biological systems (White et al., 2003). Thus, the accumulation of radiocaesium in the food chain has implications for health and the environment.

In recent years, various models for predicting the uptake of radiocaesium have been developed. Darrah and Staunton (2000) have developed a dynamic mechanistic model, based on the approach adopted by Barber and co-workers (Barber, 1984), for the simulation of the fate of Cs in soil and its uptake by root. In respect to the Barber-Claassen approach, this model introduces innovative features such as: (i) dynamics in root lifetime and exploitation of soil areas; (ii) redistribution of absorbed species within the plant and recycling back to the soil via the roots; and (iii) variation in rooting density with depth. However, the model was used to simulate the fate of radiocaesium and has not been validated with experimental data mostly because many important input parameters such as root radius, the root length that actually absorbs ions from the soil solution, and root depth distribution, are difficult to determine under field experimental conditions (Darrah and Staunton, 2000).

Established models which consider radiocaesium uptake by plant, such as ECOSYS (Müller and Pröhl, 1993) do not incorporate the effects of soil properties on the availability of radiocaesium but instead describe radiocaesium uptake from an agricultural soil (Absalom et al., 1999). A development of these models was obtained by Absalom et al. (2001) who presented a semi-mechanistic model which predicts the radiocaesium soil-to-plant transfer factor (TF, Bq Kg\(^{-1}\) plant/Bq Kg\(^{-1}\) whole soil) on the basis of easily measurable soil characteristics (clay content, organic carbon content, exchangeable K and pH). This model has been tested against independent data using a database covering contamination time periods of 1.2-10 years and accounted for 52% of the observed variation in the transfer factor.

This model has been used to predict the soil-to-plant transfer factor of radiocaesium for the tropical and subtropical environments of Bangladesh, China and Japan (Rahman and Voigt, 2004). Calculated values for rice overestimated the measured values due to an overestimation of the calculated CECs in soils in the selected regions.

As Ehlken and Kirchner (2002) pointed out, the main problem of using the TF for describing the soil-to-plant transfer of radiocaesium is its inadequacy for describing the complexities of the soil-root interface. These authors indicated that by definition the TF averages out the distribution of roots and radiocaesium in the soil and their time-dependent variations. Thus, it is not surprising that measured soil-to-plant transfer factor of radiocaesium shows ranges of up to three orders of magnitude even for individual soil-crop combinations (Frissel et al., 2002; Nisbet and Woodman, 2000).
The Barber-Claassen model might be a better tool for predicting the uptake of radionuclides, since it is a mechanistic model which combines equations describing nutrient influx in the root with equations describing root growth. This model has been successfully tested in agricultural systems for various nutrients (Barber, 1984) and heavy metals (Adhikari and Rattan, 2000; Mullins et al., 1986). Recently the model has been used to predict the uptake of radiocaesium by pea plants at different development stages (Roca-Jova and Vallejo-Calzada, 2000). Simulated and measured values corresponded for seedling and flowering stages but differed for the fructification stage.

In this paper we analyzed the possibilities of using the Claassen-Barber model to predict $^{134}$Cs uptake by maize plants grown in a Gleyic Cambisol at two plant development stages: leaf development stage 3 and pollen shed. The input parameters required in the model have been directly measured by performing various experiments as described in Barber (1984).

**Theory**

The Barber-Claassen model describes nutrient uptake by roots growing in soil. It describes the extent of the root system and its increase with time by using values for the initial root length, rate of increase in root length, and mean radius. Nutrient uptake by the root is assumed to be a function of the concentration in soil solution at the root surface as described by Michaelis-Menten equation. Supply of nutrients to the root through the soil is assumed to be by mass-flow and diffusion (Barber and Silberbush, 1984). As the root grows, uptake is calculated for each additional unit of root growth and total uptake is obtained by summarizing uptake for all root units. Hence, plant uptake is dependent both on the uptake of each root unit and the number of roots in the system. The model assumes that: (i) a root segment can exploit only a limited volume of soil given by a cylinder of radius $r_0$, that is, the average half distance of neighbouring roots; (ii) roots are distributed evenly in the whole soil volume and no allowance is given for changing distance among roots as roots grow; (iii) no changes in soil water content are assumed during the period of calculation; (iv) the soil is assumed homogeneous and isotropic; and (v) root is a sink for nutrient only, that is, neither root exudates nor their action on nutrient availability and micro-organism activity are considered (Claassen and Steingrobe, 1999). The used model was downloaded from www.gwdg.de/~uaac/download.htm (Steingrobe et al., 2000).

**Materials & Methods**

**Experimental design**

*Soil:* An untreated soil was collected from a Gleyic Cambisol (FAO, 1988) that has been under grassland and has not been ploughed for the last 30 years. Selected soil chemical and physical properties are given in
Table 4.1. The soil was taken from the 0-20cm depth of the field site and then it was air-dried at 24°C for one week. Stones and plant debris were separated and the soil was sieved at 6mm.

Table 4.1. Selected soil chemical and physical properties of the field site

<table>
<thead>
<tr>
<th>Horizon</th>
<th>Sand (g kg⁻¹)</th>
<th>Silt (g kg⁻¹)</th>
<th>Clay (g kg⁻¹)</th>
<th>Bulk density (10³ kg m⁻³)</th>
<th>Organic matter (g kg⁻¹)</th>
<th>pH</th>
<th>CEC (cmolₑ kg⁻¹)</th>
<th>Cs (µg g⁻¹)</th>
<th>K (mg g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aₜ (0-20 cm)</td>
<td>413</td>
<td>320</td>
<td>267</td>
<td>1.1</td>
<td>57</td>
<td>7.4</td>
<td>18.3</td>
<td>3.3 ± 0.7</td>
<td>10.4 ± 0.03</td>
</tr>
<tr>
<td>B (20-40 cm)</td>
<td>343</td>
<td>357</td>
<td>300</td>
<td>1.5</td>
<td>16</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>BC (&gt;40 cm)</td>
<td>318</td>
<td>44</td>
<td>279</td>
<td>1.7</td>
<td>2</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

† Based on sedigraph analysis, after 6 min of ultrasound treatment in 0.1% Na-hexametaphosphate.
‡ Schweizerische Referenzmethoden der Eidgenössischen landwirtschaftlichen Forschungsanstalten (1999).
§ pH was measured in a suspension of 1g of dry soil to 2.5mL deionized water.
| CEC: cation-exchange capacity (Thomas, 1982).
| X-ray fluorescence spectrometry.
| n.d.: not determined

On October 22, 2003, the sieved soil was divided into 16 portions of 22kg and each portion was spiked with a solution as to add 554Bq kg⁻¹ soil of ¹³⁷Cs (in chloride form and diluted in 1:100 water solution of 0.1M HCl, with a specific activity of 37MBq mg⁻¹, purchased at Amersham®). 6L of Osmosis I water of an electrical conductivity of 17µS cm⁻¹ at 25°C was added to the soil to bring the soil water content to field capacity (0.28 m³ m⁻³). To obtain a homogeneous activity, each soil portion was mixed with the solution for 3h in a rotating concrete mixer. The labelled soil was repacked in plastic pots (26cm x 36cm and 25cm deep) to reach a bulk density of 0.94 g cm⁻³. To test the homogeneity of the radioactivity a sample was taken from each pot and the activity measured by γ-spectroscopy. The coefficient of variation of these activities was 0.08 to 0.09, indicating an acceptable homogenous distribution.

Plants: On October 17, 2003, seeds of maize (Zea Mays L. cv. Corso) weighing from 0.28g to 0.30g were selected, put in 2/3 soil and 1/3 sand (0.7mm) substrate and incubated at 28°C for 3 days. Germinated seeds were transferred in a climate chamber for 2 days. Seedlings, homogenious in growth, were chosen to be transferred to the pots. On October, 23, 2003, one seedling was placed in the middle of each pot. On the same day 11 g N m⁻² as NH₄NO₃ and 4.1 g P m⁻² as KH₂PO₄ were manually applied in solid form onto the soil surface of the pots as recommended for maize production in Switzerland. Four replications were used in a completely randomized design. Acid-washed quartz sand (2-3mm) was applied to the surface to reduce evaporation. Plants in the greenhouse grew under the following conditions: 16h photoperiod,
day/night temperatures 25°C/20°C, 40-50% relative humidity of ambient air, and 300 μmol photons m⁻² s⁻¹ minimum light intensity (provided as artificial light by 400W DL/BH Lamps, Eye, Japan). The soil moisture was maintained constant at 0.28 m³ m⁻³ by weighing the pots and replenishing the water lost by adding Osmosis I water of an electrical conductivity of 17μS cm⁻¹ at 25°C. The position of the pots was regularly changed during the duration of the experiment.

On November 17, 2003, and on December 18, 2003, when plants reached leaf development stage 3 and pollen shed, respectively, the stem was cut 1cm above the soil level. The shoots were chopped and oven dried at 105°C for 24 hours. The dry weight was determined and the dry material milled to powder. A portion of the sample was used to measure the concentration of K and Ca, while the remaining part was weighted and transferred into calibrated γ-spectrometry containers to measure the concentration of ¹³⁴Cs. Soil was rinsed off from the root samples using the hydro pneumatic elutriation system, (Gillson’s Inc.) developed by Smucker et al. (1982). Roots samples were used to estimate the root parameters.

Estimation of model parameters

Soil parameters

Nutrient initial concentration in the soil solution (Cᵢ, mmol (Bq) dm⁻³): the concentration of K, Ca and ¹³⁴Cs in soil solution was determined by a water displacement experiment as described by Barber (1984). Four samples of 400g of the same soil used for the pot experiment were spiked with ¹³⁴Cs and fertilized using the same quantities and procedure described above. The samples were then incubated at field capacity (0.28m³ m⁻³) and 25°C for 3 weeks. Thereafter, samples were repacked in a 7.5-cm diameter Plexiglas column. The soil surface was covered with filter paper. The soil was equilibrated for 24h and 40 mL was added at 4mL h⁻¹ and displaced soil solution filtered through a 0.02 μm filter and analyzed for the K, Ca and ¹³⁴Cs. The retention of ¹³⁴Cs by the filter was negligible. K and Ca were measured by ICP-MS (Agilent, 7500 C) and ¹³⁴Cs by γ-spectrometry.

Buffer power (b, -): it is the ratio between the total amount of initial concentration of labile ions in the soil Cₛᵢ (mmol (Bq) dm⁻³) and ion concentration in soil solution Cᵢ (mmol (Bq) dm⁻³). It can be described by the linear equation:

\[ \Delta Cₛᵢ = b \Delta Cᵢ \]  

The soil buffer power for K is approximately linear so b would be approximately constant (Barber, 1979). The Cₛᵢ for K was determined by the method of Barber (1984) as follows: four samples of 10g (dry weight) of moist soil taken from the remaining soil of the pot experiment were shaken for 20 minutes with 100mL of 1 mol NH₄OAc/L, pH 7. The solution was centrifuged for 10 min. at 4000g s⁻¹, separated by filtration through a 0.02 μm filter and analyzed by ICP-MS (Agilent, 7500 C). The Cᵢ was obtained as described above for the fertilized soil.
Since it is not known whether the soil buffer power of $^{134}$Cs in our soils is constant a non-linear description was used for $^{134}$Cs. For a non-linear description of the buffer power, the Freundlich function was used (Steingrobe et al., 2000):

$$\Delta C_{si} = b \times \Delta C_{il}^\alpha$$

(2)

The experiment was carried out as follows: 400g (dry weight) of sieved soil were spiked with $^{134}$Cs (in chloride form and diluted in 1:100 water solution of 0.1M HCl, with a specific activity of 37MBq mg$^{-1}$, purchased at Amersham®) increasing over seven steps (193, 314, 396, 529, 655, 743 MBq kg$^{-1}$ of soil). The highest activity was chosen in relation to safety standards and the lower ones were then obtained by dilution of the standard solution. Four replicates for each $^{134}$Cs concentration were used. Sufficient water was added to increase the water content to field capacity (0.28 m$^3$ m$^{-3}$), and the soil was incubated for 3 weeks at 25°C. The concentration of $^{134}$Cs in soil solution ($C_{il}$) was obtained with the displacement column procedure described above.

The concentration of labile ions in soil solution ($C_{si}$) of each soil sample spiked with the seven $^{134}$Cs activities was obtained by extraction with NH$_4$OAc as described above for K. A similar method for the extraction of Chernobyl derived $^{134}$Cs in a loam-sandy soil was used by Rigol et al. (1999). These authors have obtained desorption rate of about 40%. In our case the exchangeable fraction represented 55% of the activity added, because of the much shorter elapsing-time (3 weeks vs. more than 10 years) between contamination and extraction in our experiment. The buffer curve was obtained by plotting the activity of extracted $^{134}$Cs against the concentration of $^{134}$Cs in the soil solution (Figure 4.1).

![Figure 4.1. Relation between extracted $^{134}$Cs and $^{134}$Cs concentration in the soil solution obtained by water displacement procedure, fitted by a Freundlich function.](image-url)
**General discussion and conclusions**

**Diffusion coefficient** \( (D_c, \text{ cm s}^{-1}) \): was calculated from the equation:

\[
D_c = D_L \cdot \theta \cdot f \cdot \frac{1}{b}
\]

(Nye and Tinker, 1977) \hspace{1cm} (3)

Where \( D_L \) is the diffusion coefficient of the solute in free solution \( (D_L=1.96 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1} \text{ for K (Roca-Jova and Vallejo-Calzada, 2000) and } D_L=2 \times 10^{-8} \text{ cm}^2 \text{ s}^{-1} \text{ for } ^{134}\text{Cs (Kirk and Staunton, 1989)}); f \) is the solid-phase impedance factor which takes account of the tortuous pathway followed by the solute through the pores \( (f=1.58^* \theta-0.17 \text{ for a soil with <75\% of sand and a water content >0.15 (Barraclough and Tinker, 1981)}) ; \) \( \theta \) is the soil moisture content and \( b \) is the soil buffer power.

**Water uptake velocity** \( (V_0, \text{ mL cm}^{-2} \text{ s}^{-1}) \): was estimated by the method of Roca-Jova and Vallejo-Calzada (2000), as follows:

\[
V_0 = \left( \frac{TCa}{Ca_i} \right) \left( \frac{1}{2 \pi r_0 L_m t} \right)
\]

\hspace{1cm} (4)

where \( TCa=\text{total Ca uptake by plants (\text{umolCa/pot}), measured by microwave digestion described below}; \)
\( \text{Ca}_i=\text{bulk soil solution Ca concentration (\text{umol mL}^{-1} \text{ measured by the water displacement experiment described above}}, \)
\( r_0=\text{average root radius (cm)}, \) \( L_m=\text{mean root length per pot (cm)}; \) \( t=\text{plant growth periods (seconds)}. \)

This method was used because Ca supply to roots occurs by mass flow.

**Plant parameters**

**Root parameters**: Root length \( (L_m) \), average root radius, \( (r_0) \), and half mean distance between roots \( (r_i) \) were measured directly on washed root samples obtained from the pot experiment and from 5-days old seedlings used at the beginning of the experiment by WinRHIZO software, version Pro 3.10b (Reagent Instruments Inc., Quebec, Canada). Root growth rate \( (k) \) was estimated as in Roca-Jova and Vallejo-Calzada (2000), as follows:

\[
k = \left( \frac{\ln L_t - \ln L_0}{t} \right)
\]

where \( L_t=\text{root length at the end of the studied growth stage (cm)}, \) \( L_0=\text{root length at the beginning of the studied growth stage (cm)}; \) \( t=\text{duration of the growth stage (seconds).} \)

**Michaelis-Menten parameters** \( (I_{\text{max}}, K_m \text{ and } C_{\text{min}}): for K and } ^{134}\text{Cs uptake were estimated in hydroponic culture using the method of Claassen and Barber (1977). Five-day old maize seedlings were obtained as described above (experimental design). Eight seedlings, homogeneous in growth, were transplanted into 10L plastic containers containing aerated nutrient solution of the following composition: 5mM of \( \text{Ca(NO}_3)_2 \), 0.15 mM of \( \text{KH}_2\text{PO}_4 \), 0.2 mM of \( \text{CaHPO}_4 \), 2mM of \( \text{MgSO}_4 \), 0.1mM of \( \text{Fe-EDTA} \), 0.05mM of \( \text{KCl} \), 0.025mM of \( \text{H}_2\text{BO}_3 \), 0.002mM of \( \text{MnSO}_4 \), 0.002mM of \( \text{ZnSO}_4 \), 0.0005mM of \( \text{CuSO}_4 \), 0.0005mM of \( \text{Na}_2\text{MoO}_4 \). Water level and pH (6.0-6.5) were kept constant throughout the growing period by changing the nutrient solution at two days interval. Plants were grown in the greenhouse under the same conditions described for the pot experiment. When plants reached either leaf development stage 3 (LDS3) (14 days old) or pollen shed (PS) (56 days old), they were transferred into new barrels after adding a target } ^{134}\text{Cs
activity concentration of 9.2 kBq (in chloride form and diluted in weakly acid solution, with a specific activity of 37 MBq mg⁻¹, purchased at Amersham®) and let it equilibrate for 10 min. Aliquots of solution (1 mL for ¹³⁴Cs and 4 mL for K) were removed manually every 10 min for 7.5 hours. After subtracting the solution samples a similar amount of deionised water was added to the barrels to maintain a constant solution volume. Root length and root radius were measured directly on root samples by WinRHIZO software, version Pro 3.1b (Reagent Instruments Inc., Quebec, Canada).

The equation (6) was used to calculate the Michaelis-Menten uptake kinetic parameters:

\[ I = \frac{I_{\text{max}} \times (C - C_{\text{min}})}{K_m + C - C_{\text{min}}} \]  

(Nielsen, 1972)  

where \( I \) is the net ion influx, \( I_{\text{max}} \) (Bq or \( \mu \text{mol cm}^{-2} \) of root s⁻¹) is the maximum influx, \( K_m \) (\( \mu \text{mol m}^{-3} \)) is the Michaelis-Menten constant, i.e., the concentration \((C - C_{\text{min}})\) where \( I \) is 50% of \( I_{\text{max}} \), and \( C_{\text{min}} \) (\( \mu \text{mol m}^{-3} \)) is the concentration where net influx is zero. The depletion data were transformed into rates of ¹³⁴Cs and K influx per unit root length.

**Sensitivity analyses**

Sensitivity analyses were conducted to evaluate the effect of the following parameters: \( b, C_b, V_0, D_e, f, I_{\text{max}}, K_m, L_m, r_0, r_1, \) and \( k \) on ¹³⁴Cs and K predicted recovery. The value of each parameter was varied by a factor of 0.5, and 2.0 and the resulting recovery predictions plotted relative to ¹³⁴Cs and K recovery under initial conditions.

**¹³⁴Cs measurement**

The concentration of ¹³⁴Cs (half-life \( t_{1/2} = 2.07 \text{y}, 475.34 \text{ keV} \)) was measured at the \( \gamma \)-spectrometry laboratory of EAWAG (Swiss Federal Institute for Environmental Science and Technology, Dübendorf, Switzerland) on high purity Ge detectors. Plant, root and nutrient solution were measured with flat crystals. Radionuclide activities were determined in Bq g⁻¹ (dry weight) and Bq mL⁻¹ for the solution samples; decay corrected to the common date of May 14, 2003, 12:00h. Measurements error was 5 to 10%. Geometry correction and calibration are based on standard solutions.

**K and Ca measurements**

Ground samples (0.250g) of shoot and seeds were weighed in plastic tubes and 2mL of nanopure water was added. To obtain a complete wetting of the dry material samples were put in an ultrasonic bath (Bandelin, SONOREX Super, RK 510, Switzerland) for 10s and then 4mL of HNO₃ (65%, Suprapure, Merck) and 2mL of H₂O₂ were added. The samples were let stay for 30 min. and then digested in the microwave oven (MLS mega 1200, Germany) using the following program: 5min at 269W and 180°C, 8 min. at 600W and 185 °C, and 7 min. at 350W and 180°C. After digestion, samples were diluted to 50mL with nanopure water and vacuum-passed through a 0.45µm Millipore membrane. The concentration of K
and Ca in the seeds, shoots, and of K in the nutrient solution was measured by ICP-MS (Agilent, 7500 C). Values of K and Ca concentrations in the seeds were subtracted from the value of plant uptake.

**Statistical analysis**

For the estimation of the buffer power for $^{134}$Cs the Freundlich function was fitted to the data by nonlinear regression. Calculations were performed in Systat® software 6.0 for Windows. For the estimation of the Michaelis-Menten parameters experimental data were fit to the relation shown in Eq. (6) by a least squares method using a nonlinear regression model. Calculations were performed in Systat® software 6.0 for Windows.

**Results and discussion**

**Soil parameters**

Values of the soil buffer power ($b$), effective diffusion coefficient ($D_e$), ion initial concentration in the soil solution ($C_{ii}$), and water uptake velocity ($V_0$) for $^{134}$Cs and K are shown in Table 4.2. The initial concentration of labile K in the soil solution ($C_{si}$) was $0.1 \pm 0.003$ mM.

Values for $b$, $D_e$, $C_{si}$, and $C_{ii}$ for K are of the same order of magnitude as the values obtained by Barber (1984) for silt loam soils fertilized with similar amount of KH$_2$PO$_4$, while the value of $V_0$ for maize plants was one order of magnitude lower in our soil. This might be due by the different methods used in measuring the water uptake velocity. In the work of Barber and co-workers the uptake velocity is calculated from water use and root surface area measurements.

<table>
<thead>
<tr>
<th></th>
<th>$b$ (-)</th>
<th>$D_e$ ($\text{cm}^2 \text{s}^{-1}$)</th>
<th>$C_{ii}$ (mmol (Bq) $\text{dm}^{-3}$)</th>
<th>$V_0$ (mL cm$^{-2}$ s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{134}$Cs</td>
<td>$331202 \pm 11.87$</td>
<td>$9.49 \times 10^{12}$</td>
<td>$1.56 \times 10^{2} \pm 1.55 \times 10^{3}$</td>
<td>$1.44 \times 10^{-7}$</td>
</tr>
<tr>
<td>K</td>
<td>$7.12 \pm 0.89$</td>
<td>$2.13 \times 10^{7}$</td>
<td>$1.4 \times 10^{-7} \pm 1.32 \times 10^{-3}$</td>
<td>$1.44 \times 10^{-7}$</td>
</tr>
</tbody>
</table>

Values of $b$ and $D_e$ for $^{134}$Cs are in the same order of magnitude to those reported by (Kirk and Staunton, 1989). For $^{134}$Cs the value of buffer power lays between the range of $10^2$ to $10^5$ which is expected for various soil types depending on the content of illitic clay minerals (Darrah and Staunton, 2000). It is not possible to compare the value of $C_{ii}$ for $^{134}$Cs with other works since no similar experiments have been carried out to estimate this value.
Plant parameters

Michaelis-Menten parameters

Michaelis-Menten parameters for the plant at pollen shed are shown in Table 4.3. To our knowledge no similar experiments were carried out with whole maize plants to determine the Michaelis-Menten kinetics parameters. Thus, it is not possible to compare our results with previous studies. Roca-Jova and Vallejo-Calzada (2000) have estimated the Michaelis-Menten absorption parameters for pea plant following the method of Seeling and Claasen (1990).

Table 4.3. Michaelis-Menten parameters for K and $^{134}$Cs measured on maize plants at pollen shed.

<table>
<thead>
<tr>
<th></th>
<th>$I_{\text{max}}$ (µmol (Bq) cm$^{-2}$ s$^{-1}$)</th>
<th>$K_m$ (µmol (Bq) cm$^{-3}$)</th>
<th>$C_{\text{min}}$ (µmol (Bq) cm$^{-3}$)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{134}$Cs</td>
<td>$1.2 \times 10^{-5} \pm 0.3 \times 10^{-5}$</td>
<td>$1.3 \times 10^{-3} \pm 0.07 \times 10^{-3}$</td>
<td>0.3</td>
<td>0.788</td>
</tr>
<tr>
<td>K</td>
<td>$4 \times 10^{-4} \pm 3 \times 10^{-5}$</td>
<td>$15.2 \pm 1.2$</td>
<td>$1.66 \times 10^{-7}$</td>
<td>0.912</td>
</tr>
</tbody>
</table>

It was not possible to measure the Michaelis-Menten parameters for the plant at leaf development stage 3 because no depletion of K and of $^{134}$Cs was obtained for this group of plants (Figure 4.2). These results are in contrast with the results obtained by Baligar et al. (1979) which report complete depletion of K and $^{137}$Cs by intact roots of 20-days-old maize seedlings. In their experiment plants were grown for 19 days at 1mM K and then transferred into a solution containing 0.185µM of K and 0.179µM of Cs. This transfer of plants from a plentiful K concentration to a limited K supply might have caused a reduction in the $K_m$ of K and thus, increased the affinity of the transport system for K. Indeed, Glass (1978) has observed that in sterile excised barley roots decreasing the concentration of K resulted in an increase in the affinity of the carrier for K.

In our experiment, plant demand for K was probably low in the plant at the LDS 3. Whereas at pollen shed, plant had a significantly higher root surface and a smaller root radius (data not shown), indicating a high production of finer roots which might be responsible for the pronounced depletion of the $^{134}$Cs and higher uptake for K.

Root parameters

Values obtained from plants at pollen shed are the following: $0.04 \pm 0.001$ cm for the average root radius ($r_0$), $0.77 \pm 0.16$ cm for half distance between root axes ($r_1$), $2241 \pm 536$ cm for root length density ($L_0$) and $1.2 \times 10^{-6}$ cm s$^{-1}$ root growth rate ($k$). These values are similar to those reported by Barber (1984) for 50 days old maize plants grown in a silt-loamy soil.
Figure 4.2. Depletion curves of $^{134}$Cs and K obtained from plants at the leaf development stage 3 (19 days old plants) and from plants at pollen shed (56 days old plants) grown in solution culture with a K concentration of 0.2 mM.

Recovery of $^{134}$Cs and K in the aerial plant parts

Since it was not possible to obtain the Michaelis-Menten parameters for plant at LDS3, K and $^{134}$Cs uptake value for plant at LDS3 could not be predicted with the model. Thus, we can only make qualitative evaluation of the application of the Barber-Claassen model for the uptake of $^{134}$Cs by maize at pollen shed. The predicted value of the recovery of $^{134}$Cs was one orders of magnitude higher than the measured value (Table 4.4), while the predicted value of the recovery of K was in the same order of magnitude of that measured but two-times higher. These results confirm that the Barber-Claassen model can successfully predict the uptake of nutrient as K, but they also indicate that it might not predict well the uptake of $^{134}$Cs. However, Roca-Jova and Vallejo-Calzada (2000) used the Barber-Claassen model to predict the recovery of $^{134}$Cs by pea plant at various development stages grown in two different soils and obtained a good prediction for the seedling and flowering stages but not for the fructification stage.

Table 4.4. Total K and $^{134}$Cs uptake measured and predicted by the Barber-Cushman model. Values refer to uptake by maize at pollen shed.

<table>
<thead>
<tr>
<th></th>
<th>$^{134}$Cs (Bq plant$^{-1}$)</th>
<th>K (mM plant$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>measured</td>
<td>0.017 ± 0.004</td>
<td>0.024 ± 0.003</td>
</tr>
<tr>
<td>predicted</td>
<td>0.26</td>
<td>0.047</td>
</tr>
</tbody>
</table>

One of the possible reasons for the overestimation of the recovery of $^{134}$Cs might be that the model does not take into account complementary-ion effects and interactions among them and thus it is not suited to predict the transfer of Cs to the aerial plant parts. It is known that Cs, presenting chemical and physiological similarities to K (Shaw and Bell, 1991; Staunton et al., 2003), compete with K for the
adsorption sites of illite and vermiculite mineral clays which are strongly selective for Cs (Maes et al., 1999) and its uptake by roots can be strongly dependent on the K concentration at the root-solution interface below a threshold concentration (Zhu et al., 2002). However, other factors might have caused the underestimation of $^{134}$Cs recovery in the aerial plant parts. For example, the indirect estimation of the water flux into the roots using Ca concentration in the soil solution and in the plant which might not be representative in calcareous or slightly calcareous soils.

**Sensitivity analysis**

**Soil parameters**

The variation factors applied to the initial concentration ($C_i$) in the soil solution had no effect on the recovery of $^{134}$Cs (Figure 4.3), while it showed significant changes in the recovery of K (Figure 4.4). Variations in water uptake velocity, diffusion coefficient, impedance factor and buffer power had no significant effect on the recovery of $^{134}$Cs (Figure 4.3), and affected minimally the predicted recovery of K (Figure 4.4). K transport to the root surface is mostly driven by diffusion (Marschner, 1995) and $b$ can strongly affect the uptake of nutrients only in the case of inter-root competition because $b$, as a component of the effective diffusion coefficient ($D_{e}$), influences the extension of the depletion zone and thereby the extent of the inter-root competition (Steingrobe et al., 2000). Thus, it is expected that these three parameters had a minor influence on nutrient transport to roots. Indeed, variations of the half distance between root axes have only slightly affected the uptake of K (Figure 4.4). Roca-Jova and Vallejo-Calzada (2000) observed also no effect of the variation of $b$, $V_0$, $f$, and $D_e$ on the predicted recovery of $^{134}$Cs. The fact that $^{134}$Cs is actively taken up by the roots through the K uptake transporter and that it reaches root surface by diffusion (Staunton et al., 2003) explains why these parameters did not affect the predicted recovery of $^{134}$Cs. Variations in the root growth constant ($k$) did not cause any change in the recovery of $^{134}$Cs and K (Figure 4.3 and 4.4). A variation of the elongation rate of roots affects the volume of soil explored by the roots but not necessarily has an effect on the absorption and uptake capacity of the growing root (Eshel and Waisel, 1996), because it is known that absorption by roots vary along a root and probably with its age, nutrient status and season (Darrah and Staunton, 2000).

**Root parameters**

Variations of the half distance between root axes ($r_i$) had no effect on the recovery of $^{134}$Cs and had a slight effect on the recovery of K. Increasing the distance between roots reduces the possibility of overlapping of the depletion zone around each root and thus competition between roots is decreased and uptake is increased. Since $^{134}$Cs is not a nutrient for the plant (Marschner, 1995) it is very unlikely that a depletion zone around roots occurs.

Increases in the average root radius ($r_0$) caused an increase in the uptake of both $^{134}$Cs and K (Figure 4.3 and 4.4).
Figure 4.3. Sensitivity analysis for the predicted $^{134}$Cs uptake. Results are expressed in relation to those obtained with the original parameters.

Figure 4.4. Sensitivity analysis for the predicted K uptake. Results are expressed in relation to those obtained with the original parameters.

The larger the roots the greater is the interface with the soil solution and so the greater is the uptake. However, this arises entirely from the greater root surface area, if a constant surface area is maintained, this effect disappears almost entirely. Thus while uptake will be enhanced by the production of additional fine roots or mycorrhizae, the size distribution of roots at constant surface area will have no significant effect.

*Root nutrient-influx parameters*

$I_{\text{max}}$ represents the capacity or potential of the root to absorb a nutrient when present in high concentrations (Jungk, 2000). Therefore, an increase of the value of $I_{\text{max}}$ caused an increase in uptake of both ions (Figure 4.3 and 4.4). However, achieving a high maximum uptake rate is possible only when a sufficient
concentration gradient can be established and maintained. Thus, the net influx remains high when no inter-root competition occurs and the concentration gradient is maintained (Claassen and Steingrobe, 1999). K\textsubscript{m} is the Michaelis-Menten constant and describes the efficiency of the root at low ion concentrations (Jungk, 2000). In the case of K the reduction of the value of K\textsubscript{m} increased the efficiency for its uptake (Figure 4.4), in agreement with the results obtained by Glass (1978). Reducing the K\textsubscript{m} value of \textsuperscript{134}Cs did not have any significant effect on the predicted uptake (Figure 4.3) probably because the value was already much low.

**Conclusions**

The model was used to predict the uptake of \textsuperscript{134}Cs and K with plants at pollen shed grown under controlled conditions and in clay loamy soil. Prediction of the uptake for \textsuperscript{134}Cs and K by maize at leaf development stage 3 was not obtained due to the impossibility to measure the Michaelis-Menten kinetic parameters. The model gave an acceptable prediction of K uptake but overestimated the uptake of \textsuperscript{134}Cs by one order of magnitude. Two possible factors are suggested to be responsible: (i) the important of complementary-ion effects and interactions among them, and (ii) dependence of the parameters’ values to the experimental methods used to measure them. It is known that at K solution concentration above 1mM the uptake of K and \textsuperscript{134}Cs occurs through a low-affinity transport system presenting channel-like properties (Véry and Sentenac, 2003), which does not display Michaelis-Menten kinetics (Tinker and Nye, 2000). Hence, one might object that the Barber-Claassen model can only be applied when the K concentration in the solution are below 1mM. Surprisingly, however, Michaelis-Menten parameters have been measured also for plants grown at high K concentration in the soil solution (Barber, 1984; Claassen and Barber, 1977). Attempt - done by the author - to measure the Michaelis-Menten parameters of maize grown in nutrient solution containing both K (1.05mM) and \textsuperscript{134}Cs have failed (data not shown), due to the impossibility of obtaining depletion curves under K luxury consumption conditions.

**Acknowledgments**

We thank Dr. Alain Mollier for helping in the experimental set-up and calculation of the Michaelis-Menten kinetic parameter and J. Leuenberger (ITOe, ETH Zurich) for helping in the establishment of the experimental set-up for the water-displacement experiment. We thank T. Rösch and T. Flura (IPW, ETH Zürich) for the ICP and microwave digestion analysis and E. Grieder (EAWAG, Dübendorf) for the γ-spectrometry measurements. T. Centofanti acknowledges the financial support of the Swiss National Foundation for Scientific Research (Project n. 3152-064135.00) and the Swiss Federal Nuclear Safety Inspectorate (HSK, Villigen-HSK, Switzerland).
General discussion and conclusions
Risk assessment models for the soil-to-plant transfer of radionuclides rely on the assumption that soils are homogenously contaminated. However, field soils are characterized by a high degree of heterogeneities mainly caused by macro-structure which can affect the distribution of surface-applied radionuclides and their accessibility by plant roots. The main objective of this thesis was to assess whether the uneven distribution of roots and the heterogeneous displacement of surface-applied radionuclides in the soil profile due to macroporous soil structure influences the soil-to-plant transfer of radionuclides. Dye tracers have been used to visualize the preferential flow paths (PFP) and as observable surrogates compounds that behave similarly to certain pollutants as pesticides and radionuclides (Aeby et al., 2001). Four radionuclides ($^{54}$Mn, $^{57}$Co, $^{65}$Zn, and $^{134}$Cs) have been applied on the surface of two untilled agricultural soils located in Switzerland and maize (Zea mays L. cv. Corso) was used as model plant. Root distribution and the spatial interrelation with the PFP have been carried out by in-situ mapping technique.

The transfer of the four radionuclides to the aerial plant parts have been analyzed on maize grown for two months in a field soil and on the same soil but sieved and homogenously contaminated before being repacked in pots (Chapter 1). Time-dependent variations of the surface-applied radionuclides and roots distribution and their influence on the transfer to the aerial plant parts have been also studied during an entire maize growth cycle (Chapter 2). The role on the uptake of radionuclides of a fraction of roots (10-15%) growing in the PFP, where most of the surface-applied radionuclides accumulated in the respect to the soil matrix, was studied in hydroponic system with a split-root experiment (Chapter 3). An attempt to model the uptake of $^{134}$Cs by maize was done using the Barber-Claassen model which combines equations describing nutrient influx in the root with equations describing root growth (Chapter 4). A synthesis and discussion of the results obtained is presented below.

**Distribution of surface-applied radionuclides and roots**

In both field experiments we observed a higher concentration of the surface-applied radionuclides in the preferential flow paths in comparison to the soil matrix, indicating that in case of heavy thunderstorm shower they infiltrate heterogeneously in the soil profile due to the structure-induced non-uniform water flow, and consequently a fraction of each radioactive element remained sorbed on the soil particles in the vicinity of the PFP (Figure 1.2, 2.2, and 2.3). In the second field experiment (Chapter 2), when areas surrounding the flow paths were sampled at radial distance of 2cm, none of the surface-applied radionuclides showed high concentration in these areas (Figure 2.2 and 2.3). This result supports those of Bundt et al. (2001) and Sinaj et al. (2002) who observed a higher radionuclide activity and P concentration sorbed on the soil surrounding the PFP.

The concentration of radionuclides at a soil depth from 15cm to 25cm when plants reached pollen shed was similar in the two field soils studied, whereas their distribution in relation to the soil depth varied in
the two field soils because of their different soil physical characteristics. It is known that macropores flow infiltration is a function of initial water content, rainfall intensity and amount, hydraulic conductivity and surface contributing area (Weiler and Naef, 2003a).

In both soils, the amount of water applied together with radionuclides and dye tracers, corresponded to a heavy thunderstorm shower. This type of irrigation was carried out in order to induce an infiltration of water within the areas of lower porosity (macropores) and in the preferential flow paths, hence allowing us to analyze the hypothesis that heterogeneous distribution of radionuclides and roots can influence their uptake. This also allowed us to study the migration of surface-applied radionuclides and dye tracers at greater soil depths. In the first experiment (Chapter 1) radionuclides were more concentrated in the upper 0-20cm of soil depth and displayed much lower concentration either in the PFP or in the matrix with depth. The high bulk density of the B horizon (Table 1.1) might have caused a reduced transport of radionuclides and dye tracers to the subsoil. In the second field experiment (Chapter 2), surface-applied radionuclides reached a depth of 40cm due to the higher saturated hydraulic conductivity of this soil and to the presence of numerous earthworm burrows. The presence of the surface-feeding earthworm *L. terrestris* forms a vertically oriented continuous network of pores which can become important pathways for a rapid infiltration (Edwards et al., 1990). Furthermore, in this field soil we observed an expansion of the PFP with time but the concentration of radionuclides in these areas decreased with time, being lower in the last harvest in comparison to the previous one (Figure 2.6 and 2.7). The expansion of the stained areas was probably due to several short-time (1-2 d) rainfall events of relative high intensities (Figure 2.8) which occurred during the six months of the experiment and most probably caused a rapid drainage through the macroporous preferential flow pathways (Figure 2.9). Thus, the radionuclides which were sorbed on the soil surface surrounding these pathways might have been transported further down in the soil profile by the flowing water. A much lower concentration of $^{65}$Zn and $^{134}$Cs in the PFP with time were obtained in comparison to $^{54}$Mn and $^{57}$Co. It is indeed known that acid leaching is very active in Zn mobilization because of increased solubility and formation of solubile complexes with organic ligands. Karathanasis (1999) investigating the potential role of water-dispersible soil colloids in transporting Zn and Cu through undisturbed soil column obtained from six different silt loamy soils, have shown that low ionic strength subsurface environments, which enhance solid-phase dispersion, moderately high pH and OC levels may significantly enhance Zn transport. Similarly, fractions of Cs can be specifically adsorbed on micaceous clay minerals, and hence can migrate with fine-dispersed suspended micaceous particles, the transport and distribution of which is controlled by the hydrological properties of the soil (Kuznetsov et al., 2001).

In both field experiments values of the root weight density were one order of magnitude higher in the upper 0-20cm of the soil profile than in the lower 20-40cm (Table 1.5 and 2.5). They also showed a slight
increase with time, although not statistically significant between pollen shed and maturity. These results are in agreement with those of Chassot et al. (2001) who found that root length density of maize grown in an untilled soil decreased strongly with increased depth and horizontal distance from the plant row.

The analysis of the overlaid root and PFP maps for both field experiments (Figure 1.3 and Table 2.7) showed that at 20 cm depth PFP covered from 6 to 11% of the analyzed area and that 10 to 15% of the roots were in the PFP. At 40 cm depth, PFP covered from 1 to 6% of the analyzed area and 3 to 6% of roots were in these areas. The number of roots occurring in the PFP increased slightly with time because of the expansion of these areas and because of the root growth and production of lateral roots. However, the ratio between percentage of roots occurring in the stained areas and percentage of stained areas was similar at both harvest occasions. Hence, the roots showed only a moderate preference for the currently active preferential flow paths. The two studied soils are loamy soils which received annually a sufficient amount of rainfall (Table 1.3 and 2.3) well distributed during the year (Figure 2.7) which maintained the water content close to the field capacity (Figure 2.8). Hence it is unlikely that prolonged dry conditions occurred, reducing the possibility of cracks and fissures formation.

In the first field experiment the higher bulk density of the 20-40 cm caused only a reduction of the root elongation rate (Pellerin and Pagés, 1994), but did not cause a clustered root growth along areas of higher porosity. Hence probably few roots used the pores created by the earthworm burrows to penetrate the soil. In our experiment it was then more a matter of chance than of a preferential growth of the roots within and around PFP.

_Uptake of radionuclides_

Measurements of the radionuclide activities in root samples obtained from the two field experiments were erroneous and unreliable due to the fine soil particle adhering on the root epidermis. Therefore, it was not possible to analyze the uptake of radionuclides by the roots grown in the preferential flow paths areas and that of roots grown in the soil matrix in the field soils.

Results obtained in the first and second chapter have shown that only a small fraction (10 to 15%) of the root system is located in the preferential flow pathways where radionuclides are concentrated in respect to the soil matrix. This suggested that the roots located in these areas were responsible for the uptake of radionuclides (\(^{134}\text{Cs}\) and \(^{57}\text{Co}\)). Hence, in this study we assessed to what extent a single root, growing in areas of \(^{134}\text{Cs}\) enrichment, may contribute to the total uptake and translocation of \(^{134}\text{Cs}\) in relation to its uptake and translocation by the whole root system growing in a homogeneously contaminated medium. It is known that the uptake of \(^{134}\text{Cs}\) occurs through the uptake system of K and that it is higher at lower external K concentration because the high-affinity transport system for K, operating at this low concentration, can not discriminate between the two ions (Zhu et al. 2002). In our experiment the uptake
of $^{134}\text{Cs}$ was higher when the external K concentration was low (0.2mM) and a single root (equivalent to 21% of the dry matter of the control root) was able to take up 50% of the amount of $^{134}\text{Cs}$ taken up by the control root; the quantity translocated to the shoots was equal to 47% of that of the control shoot (Figure 3.3). This effect was about 3 times weaker in the higher K treatment (1.05mM).

These results confirm that the K fertilization might represent a realistic countermeasure to reduce the uptake of $^{134}\text{Cs}$ by roots. Adequate K fertilization (250 Kg ha$^{-1}$ y$^{-1}$) applied after the Chernobyl accident, has proven to be one of the best countermeasures to reduce Cs contamination in grassland forage (Frissel 1996). However, it has been shown that the effect of K fertilization depended on the biological characteristics of plants and the time of the interaction of the amendments with soil. Thus, in the case of mustard and maize (leaves and stems) the addition of K fertilizers did not substantially lower Cs transfer to plants (Desmet, 1991). In our experiment the transfer of $^{134}\text{Cs}$ from the root to the shoot did not depend on the external K concentration and either in a low K concentration or in a high K concentration the shoot: root ratio activity concentration increased with time (Figure 3.7). These results might explain the observation of Desmet (1991). However, our results are in contrast with previous studies who found either a constant shoot: root ratio of various plant species with time (Staunton et al., 2003) or a decrease of the shoot: root ratio in sunflower with time (Buysse et al., 1996).

In our experiment the single root was immersed in the nutrient solution contaminated with $^{134}\text{Cs}$ for its whole length. In field soils it can be possible that roots do not grow entirely along the length of the preferential flow paths but they might explore only part of it. Since it is known that the absorption by roots vary along a root probably with its age, nutrient status and season, it can often occur that only a section of the root actually absorbs ions from the solution (Darrah and Staunton, 2000). Hence, if the absorbing portion of the root is located outside the areas of radionuclides enrichment their uptake will be probably lower.

Recovery of surface-applied radionuclides

The recovery of surface-applied $^{57}\text{Co}$ and $^{134}\text{Cs}$ in the aerial plant parts measured in the first field experiment (Chapter 1) was higher than that measured in plants grown in the greenhouse and on the same soil sieved and homogenously contaminated before being repacked in pots (Table 1.7). In relation to the total activity applied, the recovery of $^{57}\text{Co}$ and $^{134}\text{Cs}$ was 2-times and 10-times higher in plants grown in the field soil, respectively. No differences were observed in the recovery of $^{54}\text{Mn}$ and $^{65}\text{Zn}$ in the two experiments. We suggested that, since it is very unlikely that mycorrhizal fungi play any significant role in plant uptake of $^{57}\text{Co}$ and $^{134}\text{Cs}$ through the process of fungal transport, as reported by Suzuki et al. (2001) and Joner et al. (2004), they need to be located in the close proximity of the roots to be taken up in significant amounts. Thus, roots growing in the PFP might have been responsible for the higher uptake.
of the two isotopes. Contrarily, other factors might have played a role on the uptake of $^{54}$Mn and $^{65}$Zn. It is known that the uptake of Mn can be strongly influenced by the activities of rhizosphere micro-organisms as the Mn-reducing microbial group of the fluorescent pseudomonas (Posta et al., 1994), while AMF are able to take up and transport significant amounts of Zn to the roots (Bürkert & Robson, 1994). Hence for these two radionuclides rhizosphere microbial activity rather than soil macrostructure might have played a role in their uptake by roots.

A comparison of the recovery of the four radionuclides between the first field experiment (Chapter 1) and the second field experiment (Chapter 2) when plants reached pollen shed showed that the recovery of $^{54}$Mn and $^{57}$Co was similar while that of $^{65}$Zn and $^{134}$Cs was 5-times and 10-times higher in the first field experiment (Table 1.7 and 2.6). The distribution of $^{65}$Zn and $^{134}$Cs in the soil profile of the second field experiment was indeed very different from that in the first field experiment. Due to several short-time (1-2 d) rainfall events of relative high intensities, $^{65}$Zn and $^{134}$Cs which were sorbed on the soil surface surrounding the PFP and none or very small concentrations were found in the surrounding soil, might have been transported further down in the soil profile by the flowing water. Therefore, the two radionuclides have migrated downward outside the rooting zone.

Results of the study of the time-dependent variation of the recovery of the surface applied radionuclides during an entire maize growth cycle varied depending on the radionuclide. The recovery of $^{54}$Mn in the aerial part of maize increased with time showing significantly higher values at each subsequent harvest (Table 2.6). The recovery of $^{57}$Co and $^{65}$Zn in the aerial part of maize increased from the first (leaf development stage 3) to the second harvest (pollen shed), while it was constant between pollen shed and maturity (Table 2.6). $^{134}$Cs was not detectable in the aerial part of maize at LDS3 and at maturity.

Liedgens et al. (2000) have recently shown that at soil depth from 0 to 100cm, root density of maize roots, and hence the resource uptake capacities, increased until pollen shed. It is, therefore, expected that plant uptake reaches a maximum at that development stage and then stabilizes. In the case of $^{54}$Mn an increase in uptake was probably caused by the temporary flooding conditions of the soil which created a reduced environment especially in the areas of PFP. Therefore, since the solubility of Mn increases under reducing conditions (Mcbride, 1989), $^{54}$Mn became more available for the roots growing in these areas.

The decrease in the recovery of $^{134}$Cs between pollen shed and maturity was most probably caused by its convective downward transport below the rooting zone due to the facilitated colloidal transport and the presence of numerous preferential flow paths. However this reduction in the recovery of $^{134}$Cs can also be due to the recirculation of the absorbed $^{134}$Cs from the shoot and leaf to the roots (Buysse et al., 1995). Recently, Feller et al. (2000) have shown that in steam-girdling experiments where the transport of $^{134}$Cs from the xylem sap of detached wheat shoots was stopped, $^{134}$Cs was loaded into the phloem during acropetal transport.
In view of these results, it appears that the soil-to-plant transfer of radionuclides in field soils can be explained by various factors; the effect of the soil macrostructure on the distribution of radionuclides and roots represents only part of the factors influencing it. Other processes are involved in the dynamics between the soil, the root, and the radionuclides, such as: (i) environmental conditions (i.e. amount and seasonal distribution of rainfall), (ii) the activity of rhizosphere microbes and mycorrhizae fungi, (iii) the recirculation of the absorbed ions within the plant’s organs, and (iv) competing ions.

A schematic representation of the processes that are taken into account in the risk assessment model (Figure 1) as describes in the introduction and a representation of the soil-to-plant transfer of surface-applied radionuclides as revealed in this project (Figure 2) are presented below.

**Figure 1.** Schematic representation of the processes that are taken into account in the risk assessment models for the soil-to-plant transfer of radionuclides. The soil solid phase is considered a homogeneously contaminated substrate where root distribute homogenously and explore the entire soil volume. Only the competition between ions (i.e. Cs and K), the sorption/desorption processes, and the root-shoot transfer of the adsorbed ions are considered.
General discussion and conclusions

Wet deposition of radionuclides

Leaf death / Litter fall

Storage / recirculation

Translocation

Figure 2. Schematic representation of the processes influencing the soil-to-plant transfer of radionuclides in field soils as revealed in this project. The soil solid phase is characterized by two components: the preferential flow paths (PFP) and the soil matrix; the PFP are areas of radionuclides enrichment but fluxes of water and ion diffusion can occur between PFP and matrix. Chemical processes and the activity of micro-organisms and arbuscular mycorrhizal fungi (AMF) affect the availability of radionuclides; competing ions (i.e. Cs and K) affect the availability of radionuclides for their competition at the sorption sites and affect their uptake for their competition at the root uptake system. Once the adsorbed radionuclides are translocated to the aerial plant parts they can circulate back to the root or with the plant’s organs, thus affecting the recovery of radionuclides with time. Changes in the recovery of radionuclides with time can also be due to the death of leaves, to the root growth (dashed line, \(t_1\) and \(t_2\)) and to the radionuclides percolation or downward migration out of the rooting zone.

Modelling the uptake of \(^{134}\text{Cs}\) with the Barber-Claassen model

The model has been used to predict the uptake values for \(^{134}\text{Cs}\) and K by maize at leaf development stage 3 (LDS3) and at pollen shed (PS) grown in a loamy soil. At pollen shed the predicted value of the recovery of \(^{134}\text{Cs}\) was one order of magnitude higher than the measured value (Table 4.4), while the predicted value of the recovery of K was in the same order of magnitude of that measured but two-times
higher. These results confirm that the Barber-Claassen model can successfully predict the uptake of nutrient as K, but they also indicate that it might not predict well the uptake of $^{134}$Cs. Since it was not possible to obtain the Michaelis-Menten parameters for plant at LDS3 (Figure 4.2), K and $^{134}$Cs uptake value could not be predicted with the model.

Outlook and some open questions
In this project we have shown that the heterogeneous distribution of surface-applied radionuclides due to structure-induced water flow paths and their spatial relation with the distribution of roots should be taken into account in the study of the soil-to-plant transfer of surface-applied radionuclides in field soils. However, only for some of the radionuclides studied the soil macrostructure played an important role on their transfer from the soil to the plant, in other cases other factors appeared to have a major impact. In addition, the experimental conditions applied in the two field experiments might pose some limits to the applicability of this type of approach. Therefore, we suggest that further field experiments should be carried out in different environments, with various soil types, and various plant species in order to find common patterns. These types of experiments will answer the more general question: under what environmental conditions and for which type of radionuclides is the soil macro-structure an important factor for the soil-to-plant transfer of radionuclides?

Once the limits of the effect of soil structure on the soil-to-plant transfer of radionuclides are well defined it will be necessary to gain insight in the specific processes occurring at the interface between roots and PFP by carrying out single experiments focused to answer specific questions such as:

What are the chemical properties of the preferential flow paths in relation to the soil matrix in an agricultural soil? What is the microbial community living in these areas?

Is there a pattern in the type of roots growing in the preferential flow paths (i.e. only laterals, axial, seminal roots)?

Are the portions of roots growing in the preferential flow paths actually absorbing the radionuclides sorbed on the soil surface?

For what extent do roots grow in the preferential flow paths?

This approach will probably lead to a more quantitative evaluation of the effect of the soil structure on the soil-to-plant transfer of radionuclides, which might be then integrated in the mathematical models used to predict the uptake of radionuclides.


This project has been written by Dr. Achim Albrecht who supervised me during the first year of my Ph. D. For me it has been a great opportunity to work with him because during twelve months not only he introduced me to the field of radioecology which is a very interdisciplinary and complex area of research, but he also “gave” me all the enthusiasm and motivation without which I could not have finished this Ph.D.

When Achim left, the project has been directly supervised by Prof. Emmanuel Frossard and Prof. Hannes Flühler, who had to give advice, motivate and guide two students among all the other duties they had to take care of. Emmanuel and Hannes have been always available and comprehensive. In the hardest moments both have let me see the other side of the medal: Emmanuel with his easiness to take life with humour and Hannes with his great respect for the others’ work no matter how bad (or good).

Soon after I have arrived in Switzerland I have been send to France for a month to learn root mapping technique from Dr. Sylvain Pellerin and Dr. Alain Mollier (INRA Bordeaux). For me it has been an important learning experience which developed in a fruitful scientific collaboration. Though physically far away, Sylvain and Alain followed my project closely and in a couple of face-to-face discussions they helped me to take one of the possible ways that a Ph.D. student sometimes have in front of himself.

This project is composed of two sub-projects: one on the uptake and transfer of surface-applied radionuclides developed by myself, and one on the interrelation between the distribution of surface-applied radionuclides and roots in the soil profile developed by Robin Penfield. Robin and I have been working together since July 2001. We started making experiments out in the field spending entire days counting roots or taking pictures of soil profiles with the CDD camera, but in the last year each of us tried to understand specific aspects related to our part of the project, nevertheless we have continued to help each other. Ours was a small team but big enough to give us the opportunity to learn what means to work with someone else who - despite globalization - does not speak your language, does not eat the same food and did not read the same exercise book. Sometimes it was hard but we took the discrepancies as part of the game.

Two other groups of people have been extremely helpful and with their know-how have become indispensable. Jörg Leungenberger and Hannes Wydler have technically supported the field experiments and a couple of lab experiments. They always have shown a great amount of patience and coped with all our program’s changes and modification of technical aspects of the experiments.

The second group of people that played a crucial role in this project is represented by: Thomas Anken, Erwin Grieder, Thomas Flura, Theres Rösch, and Richard Ruh. The fact that unites them together is that they all helped me in the experimental part of the project. Thomas Anken allowed us to “contaminate” one of their plots at the FAT, Tänikon, and provided us - always in a very prompt way - all the information that we asked him about the field soil. This was for me a very important help which saved me a lot of
time. Erwin Grieder spent weeks in the basement of the EAWAG to measure the samples that I brought him in big amounts. I am very grateful for the amount of work that he carried out and for the reliability of his measurements. Theres Rösch and Thomas Flura did not spend weeks in a basement but they also measured and will measure many samples of mine. They did the measurements and thereafter came to me with excel files ready to be analyzed. They did more than what they were supposed to do but they never complained with me and I appreciated it a lot. Without Richard Ruh I would have spend months to optimize the hydroponic system and I would have had a heart attack for the many problems that we had in the functioning of the greenhouses.

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In 2001 we started a joint project with Prof. Nadia Goncharova form the International Sakharov Environmental University, Minsk, Belarus. Two students Ekaterina and Vyktoria decided to get involved in the project and came to Zurich few times for a couple of months. Our scientific collaboration developed in a good friendship which did not vanish after the end of the project.

It was a great experience for me to work with you, thank you very much.

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