

Shoot Cadmium Accumulation of Maize, Sunflower, Flax, and Spinach as Related to Root Physiology and Rhizosphere Effects

Dissertation

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Christos Stritsis

born in **Goumenissa, Greece**

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1. Name of referee: Prof. Dr. Norbert Claassen
2. Name of co-referee: Prof. Dr. Norbert Lamersdorf

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«Η φύσις δ' αεί προς το βέλτιστον ορμά»

Theophrastus (371–286 BC), *the causes of plants* A.16.11

The father of Botany

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LIST OF ABBREVIATIONS

C_{L0}	Concentration at the root surface
C_{Li}	Initial soil solution concentration
FAAS	Flame atomic absorption spectroscopy
GFAAS	Graphite furnace atomic absorption spectrometer
I_n	Net influx $\text{mol cm}^{-2} \text{s}^{-1}$
NST 3.0	Mechanistic model of nutrient uptake form soil by plants
ρ	Proportion of Cd translocated from the root to the shoot in %
PAR	Photosynthetically active radiation in $\text{mol m}^{-2} \text{s}^{-1}$
r_0	Root radius in cm
RA	Root-surface area per plant in cm^2
RA / SDW	Root area / shoot dry weight ratio in $\text{cm}^2 \text{g}^{-1}$
RGR_s	Relative growth rate of the shoot in s^{-1} or d^{-1}
r_i	Half distance between neighbouring roots in cm
RL_v	Root length density in cm cm^{-3}
RSDW	Relativ shoot dry weigth in %
SDW	Shoot dry weight in g
t	Time
U	Shoot Cd content in mol plant^{-1}
X	Shoot Cd concentration in mg kg^{-1} or mol g^{-1}
α	Root absorbing power in cm s^{-1}
D_L	Diffusion coefficient in water in $\text{cm}^2 \text{s}^{-1}$
θ	Volumetric soil water content in $\text{cm}^3 \text{cm}^{-3}$
f	Impedance factor

ABSTRACT

Plants differ widely in Cd accumulation when grown on Cd contaminated soils. This is because several soil properties interact with several plant properties. Plant factors determining the shoot Cd concentration of plants are the root-shoot ratio [surface area (RA) per unit of shoot dry weight (SDW)], the relative shoot growth rate (RGR_s), the Cd total net influx (I_{nt}) and the proportion of absorbed Cd that is translocated from the root to the shoot (p). And the Cd influx is furthermore influenced by plant properties like the Cd uptake kinetics and the ability of the root to affect the Cd solubility in the rhizosphere.

The aim of this research was to assess the significance of the above mentioned plant properties for the shoot Cd accumulation for different plant species. A growth chamber experiment was carried out with maize, sunflower, flax, and spinach growing on a sandy soil to which 0, 14 and 40 $\mu\text{mol Cd kg}^{-1}$ were added resulting in a Cd soil solution concentration of 0.04, 0.68 and 2.51 $\mu\text{mol L}^{-1}$. At the high Cd addition (40 $\mu\text{mol kg}^{-1}$) SDW of flax and spinach was significantly decreased by 20% and 40%, respectively while maize and sunflower showed no yield depression. The shoot Cd concentration of plants increased in all species linearly with the Cd addition to the soil. The Cd concentration in shoots varied between maize and spinach by a factor of about 30, sunflower and flax were in between. The root-shoot ratio (RA/SDW) varied by a factor of two and the RGR_s by a factor of 1.5. The main factor related to different Cd concentrations in shoots was the Cd shoot influx which varied between maize and spinach by a factor of almost 40 at low and by a factor of 26 at high Cd addition. Differences in Cd influx among species may be based on their ability to solubilize Cd in soil or on differences in Cd uptake kinetics.

Plants had a strong effect on Cd concentration in soil solution, C_{Li} , i.e. plant growth decreased (maize, sunflower) as well as increased (flax, spinach) C_{Li} . The decrease of C_{Li} at low Cd addition was not related to Cd uptake by the plants, since maize with the lowest Cd uptake showed the strongest decrease of C_{Li} (about 65%), whereas spinach with the highest Cd uptake, showed the least decrease of C_{Li} (about 20%) indicating a Cd immobilization in soil caused from root activity of maize or spinach. In contrast to decreasing, C_{Li} was increased by flax at both Cd levels up to 100% and by spinach by 23% at the high Cd addition to the soil. Plants therefore solubilized more Cd as was taken up by them. However, these effects did not totally explain the observed differences in their Cd influx. Further explanations were sought by using mechanistic modeling to simulate Cd uptake from soil. Modeling allowed including not only the effect plants had on C_{Li} but also their different Cd uptake kinetics. Uptake kinetics was described by the root absorbing power, α , which is the slope of the uptake isotherm, which is almost constant in the low concentration range as found in the soils of this experiment.

The simulated Cd influx was always higher than the measured one and for maize and flax it was about 10 times higher. The sensitivity analysis showed that the Cd soil solution concentration, C_{Li} , should be reduced by 90% for maize and by 37% for spinach in order for the simulated Cd influx to become the same as the measured one. The changes of the α value lead to similar results.

The sequential Cd fractionation in the soil with and without plants demonstrated that plants affect the Cd binding in soil. For spinach and sunflower 70% of Cd was found in the mobile and easily mobilizable fraction while for flax it was only 55%. In Mn oxides occluded Cd decreased in spinach and sunflower between 5-10% and in organically bound Cd increased in maize and flax about 5%. The other fractions contained only

5% of the total cadmium that was bound to solid phase. However, the effect different plants had on Cd binding in soil was not related to the effect they had had on Cd concentration in the soil solution.

ABSTRACT AUF DEUTSCH

Pflanzen unterscheiden sich in der Cd-Aufnahme und -Akkumulation, wenn sie auf mit Cd kontaminierten Böden wachsen. Dies ist darin begründet, dass mehrere Pflanzeigenschaften mit mehreren Bodeneigenschaften interagieren. Der Cd-Gehalt im Spross (X_s) hängt ab von: a) der Größe des Wurzelsystems, charakterisiert durch das Wurzel-Spross-Verhältnis, WSV (RA/W_s , Wurzeloberfläche zu Sprossgewicht, b) der relativen Wachstumsrate des Sprosses (RGR_s), c) dem Netto-Influx ($I_{nt,s}$) und d) dem Anteil des aufgenommenen Cd, der in den Spross verlagert wird (p). Der Cd-Influx hängt u.a. ab von der Cd-Aufnahmekinetik und der Mobilisierung des Cd im Boden durch die Wurzel.

Ziel dieser Arbeit war es, die Bedeutung der oben genannten Pflanzeigenschaften für die Cd-Akkumulation im Spross verschiedener Pflanzenarten zu untersuchen. Hierfür wurde Mais, Sonnenblume, Öllein, und Spinat auf einem sandigen Boden (pH_{CaCl_2} 4,5, 2,8% C, 5% Ton) mit drei Cd-Stufen (0, 14 und $40 \mu\text{mol kg}^{-1}$ Boden, was in einer Cd-Konzentration in der Bodenlösung von 0,04, 0,68 und $2,51 \mu\text{mol L}^{-1}$ resultierte) angezogen. Bei der hohen Cd Zufuhr ($40 \mu\text{mol kg}^{-1}$), wurde die Trockenmasse, TM, von Öllein und Spinat signifikant um 20% bzw. 40% reduziert während Mais und Sonnenblume keine TM-Depression zeigten. Der Cd-Gehalt im Spross erhöhte sich in allen Pflanzenarten linear mit steigender Cd-Zufuhr zum Boden. Er variierte zwischen Mais und Spinat um den Faktor 30, Die Cd-Gehalte von Sonnenblume und Öllein lagen dazwischen. Das WSV variierte um den Faktor 2 bis 3, wobei Spinat den niedrigsten Wert hatte und die RGR_s variierte um maximal 1,7. Die Variation in dem WSV und der RGR_s konnten die Unterschiede im Cd-Gehalt der Pflanzenarten nicht erklären. Es war

vor allem der Cd-Sprossinflux, der zwischen Mais und Spinat bis um den Faktor 40 variierte, der die unterschiedlichen Cd-Gehalte im Spross bestimmte. Der unterschiedliche Cd-Influx könnte darin begründet sein, dass Pflanzen Cd im Boden unterschiedlich stark lösen bzw. mobilisieren oder eine unterschiedliche Cd-Aufnahmekinetik besitzen. Pflanzen hatten einen starken Einfluss auf die Cd-Konzentration in der Bodenlösung, C_{Li} . Mais und Sonnenblumen erniedrigten Spinat und Öllein erhöhten sie. Die Abnahme von C_{Li} bei niedriger Cd-Zufuhr war nicht in der Cd-Aufnahme durch die Pflanzen begründet, da Mais mit der niedrigsten Cd-Aufnahme die stärkste (ca. 65%), dagegen Spinat mit der höchsten Cd-Aufnahme die geringste Abnahme von C_{Li} (ca. 20%) zeigte. Dies deutet auf eine Cd-Immobilisierung im Boden durch Mais bzw. Spinat hin. Im Gegensatz zur Abnahme, wurde C_{Li} durch Öllein bei beiden Cd-Zugaben um bis zu 100% und von Spinat um 23% bei der hohen Cd-Zufuhr zum Boden erhöht. Pflanzen mobilisierten daher mehr Cd als sie aufnahmen. Diese Effekte konnten die beobachteten Unterschiede im Cd-Influx jedoch nicht vollständig erklären. Mit Hilfe einer mechanistischen Modellierung der Cd-Aufnahme aus dem Boden wurde neben der Wirkung der Pflanzen auf C_{Li} auch deren Aufnahmekinetik berücksichtigt. Die Aufnahmekinetik wurde mit der Wurzel-Aufnahmefähigkeit (root absorbing power), α , beschrieben, welche das Steigungsmaß der Aufnahme-Isotherme ist, die, im niedrigen Konzentrationsbereich, nahezu konstant ist. Der mit dem Modell berechnete Cd-Sprossinflux war stets höher als der gemessene, bei Mais und Öllein sogar um den Faktor 10. Die Sensitivitätsanalyse zeigte, dass C_{Li} , um 90% bei Mais und um 37% bei Spinat verringert werden müsste, um eine Übereinstimmung des gemessenen mit dem berechneten Cd-Sprossinflux zu erreichen. Die Veränderung von α -Werten führte zu ähnlichen Ergebnissen.

Die sequentielle Fraktionierung von Cd im Boden mit und ohne Pflanzen zeigte, dass die Pflanzen die Cd-Bindung im Boden beeinflussten. Bei Spinat und Sonnenblume wurden 70% des Cd in der mobilen und leicht mobilisierbaren Fraktion gefunden, während es bei Öllein nur 55% waren. Die in Mn-Oxiden okkludierte Fraktion verringerte sich bei Spinat und Sonnenblume zwischen 5-10% und die organisch gebundene Fraktion stieg bei Mais und Öllein um etwa 5% an. Die restlichen Fraktionen enthielten nur 5% des gesamten Cd des Bodens. Die Wirkung der Pflanzen auf die Cd-Bindung im Boden stand jedoch in keiner Beziehung zu deren Wirkung auf die Cd-Konzentration in der Bodenlösung.

1 GENERAL INTRODUCTION

The uptake of nutrients from the soil is a complex process and depends on plant characteristics, soil properties and element properties. Therefore, many scientists have researched the basics of nutrient transport in the soil (mass flow, diffusion), nutrient dynamics in the rhizosphere (depletion, accumulation), and the uptake kinetics (Barber, 1962; Jungk and Claassen 1997; Buhse, 1992, Steingrobe, 1992, Meyer, 1993; Trehan und Claassen, 2000, 2001 und 2004; Bhadoria et al., 2004).

This study will focus on the non-essential and toxic heavy metal Cadmium (Cd). Cadmium is the 64th most abundant elements in the Earth' crust (Sarkar, 2002), and the plants grown on Cd-contaminated soils may absorb Cd in excessive quantities, which in turn, may cause clinical problems both to animals and to humans. It is therefore important to know the causes that lead to enrichment of Cd in plants.

1.1 Cadmium in soil

It enters the environment through industrial and metallurgical processes but mainly through the application of Cd-containing sewage sludge or by the application of phosphate fertilizers, which are distributed on land (Jackson and Alloway, 1991; di Toppi and Gabbrielli, 1999). Long-term applications of lands with sewage wastes containing heavy metals have been reported to affect adversely soils, plants and watercourses (Sikka et al., 2009). The high persistence of Cd in agricultural soils even after many years of application makes it a matter of concern because it is taken up by plants and through the food chain it reaches to humans and animals (Azad et al., 1986; Hooda et al., 1997; Yang et al. 2004). The

geological presence of Cd in agricultural soils is only in very small quantities (0.1 mg kg^{-1}). The Cd content of uncontaminated soils in Germany is between $0.1 - 0.6 \text{ mg kg}^{-1}$ soil, while contaminated soils show greatly increased Cd levels from 3 up to 200 mg kg^{-1} (Scheffer / Schachtschabel, 2002, p.378).

The transfer of heavy metals such as Cd from soil to plants depends on soil, plant, and metal properties. Some factors that have been suggested to contribute to differential Cd accumulation include: high Cd soil concentration; high mobilization or immobilization of Cd in the rhizosphere by root exudates; high translocation of Cd from root to the shoots; evolving tolerance mechanism (Herms and Brümmer, 1980; Harter, 1983; Bergmann, 1993, pp355 - 361; Marschner, 1995, pp537 - 595; Eriksson and Söderström, 1996, Marschner und Römheld, 1996). Cadmium is dissolved in the soil solution and bound to the solid phase. In the solid phase, Cd is present as exchangeable ion or bound on the soil substance, such as Fe and Mn oxides or organic matter. A sequential fractionation is used to characterize the different binding forms of Cd in soil (Tessier et al., 1979; Gerth et al., 1981; Mahler et al., 1982; Brümmer and Herms, 1983; Zeien and Brümmer, 1989; Zeien 1995). In the soil solution, Cadmium is present as Cd^{2+} ion, or as inorganic or organic complex (Nolan et al., 2003). The solid-solution equilibrium determines the effective Cd^{2+} concentration and this in turn the extent of complex formation with organic and inorganic ligands in the soil solution. At low pH values Cd is found as CdSO_4^0 or CdCl^+ and in the alkaline range as CdHCO_3^+ , or CdCO_3^0 CdSO_4^0 (Sponito, 1989, p69; Gerke, 1995; Helmke, 1999). The organic Cd complexes in soil solution are less characterized, because the organic ligands can be very diverse, and could come from both the soil organic matter and from root exudates. The Cd availability has a high importance for its uptake and the root effect on the Cd

solubility in soil can affect the Cd availability causing an increase or decrease of the Cd activity in the rhizosphere (i.e. the effective Cd concentration). Mench und Martin (1991) reported that the root exudates of *Zea mays* could not increase the Cd concentration in soil in comparison to root exudates of *Nicotiana spp.* due to the lower metal-binding ability of *Zea mays* exudates. The mobilization of Cd by *Nicotiana spp.* root exudates caused a linear increase of Cd concentration in the plant. Humic acid in soil can form complexes with Cd and therefore lower Cd uptake (Cabrera et al. 1988). On the other hand, organic acids such as citrate seem to be less effective and the presence of Cd-citrates in the solution does not increase shoot Cd accumulation (Senden et al., 1995; Egle, 2002). A negative effect on the Cd availability for example, could be the formation of complexes with phytochelatin. It is known that plants exposed to Cd respond with an increased production of phytochelatin in the cell, which can bind and detoxify Cd (Sanità di Toppi und Gabrielli, 1999; Keltjens und Beusichem, 1998; Wojcik und Tukiendorf, 2005; Guo und Marschner, 1995). Whether phytochelatins or similar Cd complexing agents are exuded by roots and influence the Cd activity in the rhizosphere is poorly understood and further investigations are required to explain differences in profile of root exudates among plant species to explain the effect on Cd solubility. Therefore, in this study we aim to examine the effect of plants on Cd solubility in the rhizosphere.

1.2 Shoot Cd accumulation and threshold level of damage

Cadmium toxicity in plants has been reported worldwide, which often limits plant growth and results in decreased yield. The toxicity depression occurred at different soil Cd concentration. Cadmium-sensitive plants such as spinach, soybean, and lettuce grew at loamy soil (pH 7.5) pre-treated with a municipal

sewage sludge were injured by soil Cd levels of 35.5 - 115.5 $\mu\text{mol Cd kg}^{-1}$; whereas, tomato and cabbage tolerated soil levels of approximately 1.5 mmol Cd kg^{-1} soil without exhibiting injury symptoms. Rice was tolerant at 5.6 mmol Cd kg^{-1} soil (Bingham et al., 1975). Keller (2000) reported that *Lolium multiflorum* and *Trifolium pratense* grew well on a sandy soil containing 11.5 $\mu\text{mol Cd kg}^{-1}$ (pH 5; C% 3.2; Clay 5%) but a silty soil containing 14.3 $\mu\text{mol Cd kg}^{-1}$ (pH 6.4; C% 1.1; Clay 20%) showed a dry matter decrease of 30% and 20%, respectively. On other polluted silt soil, containing 231 $\mu\text{mol Cd kg}^{-1}$ (pH 6.9; C% 0.6; Clay 18%) a yield reduction of 65% in ryegrass and 72% in red clover were found. On the other hand, in the same experiment *Lupinus albus* increase of the biomass of 40% 231 $\mu\text{mol Cd kg}^{-1}$ and caused a positive effect on plant growth while *Spinacia oleracea* had produced 5 fold less dry matter at 231 $\mu\text{mol Cd kg}^{-1}$ than as at 11.5 $\mu\text{mol Cd kg}^{-1}$ soil. To ensure a safe agricultural production the critical level must be known at which a plant is able to grow without effect on yield and quality.

However, threshold value varies significantly in different plants. Healthy plant growth is associated with the Cd concentration in the plant. Plant species accumulate varying quantities of Cd in shoot not only in different species of a genus but also among genotypes within a species (Padeken 1998; Keller 2000; Egle 2002; Dunbar et al., 2003). Egle (2002) concluded that *Lupinus albus* had the lowest Cd shoot concentration (0.2 mg kg^{-1}) compared to those of *Lupinus angustifolius* (0.6 mg kg^{-1}) and *Lupinus luteus* (0.9 mg kg^{-1}) grown on sandy soil (pH 5.4) with 5.3 $\mu\text{mol kg}^{-1}$ total Cd concentration. In the same experiment it was remarkable the high Cd concentration of *Lolium multiflorum* which measured 5 times higher than in *Lupinus albus* and from 1.5 to 2.5 times higher than in *Lupinus angustifolius* and *Lupinus luteus*. Results of Keller (2000) showed that at 11.5 μmol

Cd kg⁻¹ and at 14.3 μmol Cd kg⁻¹ *Lolium multiflorum* and *Trifolium pratense* with 1.5-3.5 mg kg⁻¹ had a low Cd shoot concentrations, while at 231 μmol Cd kg⁻¹ obtained high Cd shoot concentrations of 11-13 mg kg⁻¹. Shoot Cd concentration of two spinach genotypes (Monnopa and Tabu) was from 9 to 48 mg kg⁻¹ significantly higher than those of lupine. Furthermore, Cd concentration in sunflower kernels of commercial non-oilseed hybrids grown at slightly contaminated soil (3.5 μmol Cd kg⁻¹) varied from 0.79 to 1.17 mg kg⁻¹ (Li et al. 1995).

Cadmium toxicity symptoms are puny growth and yellowing of the intercostal fields to necrosis of tissues and characteristic brown surfaces on the leaves and leaf fall (Barcelo & Poschenrieder; Salt et al., 1995; Dong et al., 2005). Cadmium probably has a negative effect on the process of photosynthesis, by reducing the chlorophyll content (Hassan et al., 2005), and reducing of transpiration (Haag-Kerwer et al., 1999). Similarly, Cd has an inhibitory effect on the activity of various enzymes (Weigel and Jäger, 1980; Uraguchi et al., 2006), on the functionality of membranes (Kennedy and Consalves, 1987), as well inhibits chlorophyll biosynthesis (Stobart *et al.*, 1985; Van Assche & Clijsters, 1990; Somashekaraiah *et al.*, 1992). Moreover, Liu et al. (1995) reported damage in the nucleotides via Cd. The appearance of chlorosis due to Cd supply was also observed in other species (Alcantara et al., 1994), which linked it, to a decrease in net assimilation rate (NAR) for barley (Vassilev et al. 2002; Hassan et al. 2005). Sandalio et al. (2001) reported that the growth inhibition of pea plants was accompanied by a significant decrease in the photosynthesis rate. Antagonistic effects between Cd and Zn, Cu, Mn and Ca during the uptake and accumulation was described by Yang et al.

(1996) for cereal crops which could also explain the above-mentioned damage symptoms.

Cadmium accumulation in plants has been studied in several species. In most of the plant species, much of the Cd taken up by plants is retained in the root and its translocation to shoot is low. In general, all plants showed higher Cd concentrations in the roots than in shoots. Roots accumulate much greater amounts of Cd than shoots and dicotyledonous absorb more Cd from solution than monocotyledon (Kuboi et al.1986, Inouhe et al 1994). In *Lupinus albus* is about 15 mg Cd kg⁻¹ strongly accumulated in roots and only 0.2 is transported to the shoot system. *Lupinus angustifolius* absorbed Cd from the rooting medium (0.5 mg Cd kg⁻¹ soil) and the localization of absorbed Cd is greater in roots (11.5 mg Cd kg⁻¹) than in shoots (0.7 mg Cd kg⁻¹) (Egle 2002). From the above it is understood that root system of plants act as a first barrier of Cd translocation to the shoot and so the symptoms and biomass reduction appear in the plant at different Cd shoot concentration.

For this study maize, sunflower, flax and spinach species were selected because they showed very different behavior in the study of Padeken (1998). While the plants grew on the same silt soil (pH 7.4; C% 1.6; Clay 18%; Cd 89 µmol kg⁻¹) obtained maize (0.62 mg Cd kg⁻¹) and sunflower (1.99 mg Cd kg⁻¹) low shoot Cd concentration while flax (4.87 mg Cd kg⁻¹) and spinach (6.22 mg Cd kg⁻¹) had higher shoot Cd concentration among 13 species tested. At the same Cd concentration extracted with 0.1M CaCl₂ (3.4 µmol Cd kg⁻¹) spinach showed a higher Cd uptake (269 ng cm⁻²) than maize (17 ng cm⁻²), so the roots of spinach have a higher activity to use the Cd concentration in the rhizosphere, resulting in a higher Cd

uptake per unit root. This result indicates that spinach root may increase the Cd concentration in the rhizosphere leading to that higher Cd uptake per unit of root.

The accumulation of essential heavy metal, like Mn and Zn in the shoot depends on the uptake. The heavy metal uptake, in turn, depends on the size of the root system and the influx (Sadana et al., 2003). However, for non-essential heavy metal that is valid for a certain area of Cd concentration in soil in which the physiology of root system is not adversely affected. In this study, we want to focus on the difference in Cd uptake of the plant species mentioned above and to determine the effect of soil solution concentration on plant growth and accumulation of Cd under lower ($< 10 \text{ mg kg}^{-1}$) Cd content of the soil. In the experiments we used both monocots and dicots plant species. In the selection of these plants was regarded not only their differences in Cd shoot accumulation, but also their use after harvest. As for industrial plants their cultivation on contaminated soils could be acceptable, the cultivation of non-industrial plants would be dangerous for fresh consumption. For the risk to humans and animals are particularly important in Cd concentrations in the usable plant organs, i.e. these are often the seeds that have been studied on their heavy metal concentrations.

Maize as a grain and a C_4 plant is cultivated after wheat and rice as the world's third most important grain, and is a staple food in many countries and an important forage plant. In Germany, seventy percent of the cultivated area of maize used for animal feed and the residual processed in the food industry.

The flax oil consists mainly of unsaturated fatty acids (18 C) such as oleic acid (C 18:2) and linoleic acid (C 18:3) that are in demand for the production of

pharmaceuticals and cosmetics (van den Berg et al., 2004). The ripe dried flax seed and the cold-pressed oil used in nutrition and the manufacture of paints.

Sunflower seeds are used in bread and, above all, the sunflower oil is used as a plant whose oil is very valuable for human consumption. The sunflower oil is rich in oleic acid (C 18:1), a long-chain monounsaturated fatty acid (Flagella et al., 2002), which is of particular importance for the chemical industry.

Spinach is one of the important vegetables grown on soil and can be eaten as a salad as well as a large part of the spinach is used in the food industry as a deep-frozen product on the market. Spinach has a high content of minerals (Fe, Zn, Ca, K, Cu, Mg, Na, P), vitamins (β -carotene, also known as vitamin A, vitamin B group, vitamin C), fiber, fat and protein.

1.3 Factors affecting the shoot Cd concentration of plants

Shoot Cd concentration is dependent on:

- a) root surface area (RA in cm^{-2}) per unit of shoot weight (SDW in g),
- b) the relative shoot growth rate (RGR_s),
- c) the Cd total net influx (I_{nt} in $\text{mol cm}^{-2} \text{s}^{-1}$) and
- d) the proportion of absorbed Cd that is translocated from the root to the shoot (p).

Often only uptake into the shoot is determined and the influx may be named as net shoot influx (I_{ns}) notice that $I_{ns} = I_{nt} \times p$

All these factors can enter mathematically into the following equation (Ney and Tinker 1977):

$$\text{Shoot Cd concentration (X)} = \frac{RA}{SDW} \cdot \frac{1}{RGR_s} \cdot I_{nt} \cdot p$$

Keller (2000) found that the shoot Cd concentration of various types of spinach was positive correlated with RA/SDW but in comparison of different species was not given this correlation, i.e. different Cd levels between species were due to differences in the influx (Egle, 2002). Cadmium influx depends on the uptake kinetics and the effect of the root on Cd activity (i.e. the effective Cd concentration) in the soil solution. The uptake kinetics as a factor of Cd influx from the soil into the root has not been investigated to our knowledge, as is generally considered that significant for heavy metals such as Cd is the low availability in the soil for the influx. The previous results indicate that differences in Cd uptake from soil among species may depend on different uptake kinetics and on the way that the root affects the solubility of Cd in the rhizosphere. Differences in influx are related to the uptake kinetic parameters I_{\max} and K_m (Michaelis - Menten kinetics), which result from plants adaptation to Cd concentration (Jungk and Claassen, 1997; Claassen and Steingrobe, 1999; Jungk, 2002). Michaelis - Menten kinetics may not unlimited apply to characterize the Cd uptake kinetics by plant because there is no point of determining the maximum net influx by adding unrealistic high levels of Cd to a nutrient solution or soil as Cd is not an essential but toxic element.

The uptake kinetics is the relationship between the net influx (I_n) and the concentration at the root surface, C_{L0} , which is in a wide range of concentrations a saturation function (Jungk and Claassen, 1997).

In the lower concentration range, this relationship is approximately linear for both Cd as well as micro and macro nutrients (Sadana et al., 2005), and can be described as follows:

$$I_n = \alpha \cdot C_{L0}$$

Where α describes the root absorbing power, in cm s^{-1} (Nye 1973) and it is the slope of the uptake isotherm (which can be estimated from the ratio of I_{max} / K_m or measured directly, i.e. I_n / C_{L0}) at the very low concentration. The α value makes comparisons among nutrients and plants easier.

Sadana et al., (2005) reported α value of Mn $1.1 \times 10^{-6} - 1.7 \times 10^{-6} \text{ cm s}^{-1}$ for three wheat cultivars and Zahan (2008) α value $26.2 \times 10^{-6} - 41.6 \times 10^{-6} \text{ cm s}^{-1}$ for four bread wheat cultivars. For macronutrients, Claassen (2006) mentioned α value of K ($100 \times 10^{-6} - 1000 \times 10^{-6} \text{ cm s}^{-1}$ for wheat) and P ($200 \times 10^{-6} - 1000 \times 10^{-6} \text{ cm s}^{-1}$ for oil seed rape). These values show that root absorbing power varies very strongly and according to equation $I_n = \alpha \times C_{L0}$ at the same concentration at the root surface the influx of P or K will be much higher than of Mn. This aspect of uptake kinetics has not yet been considered for Cd and it is important to find how high Cd concentrations in plants are caused by high α values.

The influx also depends on how much Cd the soil can supply to the root, i.e. it depends on the transport properties of the soil. The result is a combination of plant and soil properties that influence finally the Cd influx. Therefore, simple relationships often cannot explain observed phenomena. To overcome this problem, several authors have used mathematical simulation to improve the understanding of the complexity involved in the process of nutrient uptake by plant roots growing in soil (Claassen et al., 1986; Claassen and Steingrobe, 1999).

These models are based on movement of nutrients from soil to roots by means of mass flow and diffusion and on nutrient-uptake kinetics, mostly following Michaelis - Menten kinetics. The model will help to understand why plants differ in their Cd accumulation because besides the calculated Cd uptake by plants, the Cd dynamics in the rhizosphere is calculated. Therefore, it will be possible, for example, to recognize to which extent different species lower the Cd concentration in the rhizosphere. Furthermore, we aim to evaluate the input parameters that influenced Cd uptake and transport and to assess the importance of various parameters in Cd uptake.

1.4 Investigation of hypotheses in this research

The conclusion from the previous is that Cd shoot accumulation is dependent on Cd uptake. The Cd uptake in turn depends on the size of the root system and the Cd influx. In this work we want to focus mainly on the difference in Cd influx of different plant species grown in soil and to study the following hypothesis.

- Cadmium influx is strongly affected by the Cd uptake kinetics (α -value, root absorbing power).
- Cadmium influx can be quantitatively described by a deterministic model (NST 3.0).
- Cadmium influx is influenced by rhizosphere effects (Cd solubilization in the rhizosphere).

2 SHOOT CADMIUM CONCENTRATION OF SOIL GROWN PLANTS AS RELATED TO THEIR ROOT PROPERTIES

2.1 Introduction

Cadmium (Cd) is the 64th most abundant elements in the Earth' crust (Sarkar, 2002), and the study of Cd uptake by plants has been done because of its potential risk for human health through the food chain. To assess the risk it is necessary to know the plant Cd concentration and to which extent it is influenced by factors such as soil properties (pH, clay content, humic substances, buffering capacity, water content, Cd content in soil, Eh) and plant properties (root activity, uptake, tolerance or sensitivity, translocation from root to shoot, plant age, parts of the plant). The Cd uptake of plants depends for one on Cd availability in soil, and for the other it differs among species, and among genotypes of the same species and therefore Cd accumulation in the shoot varies greatly (Wang 2002, Simmons et al., 2003). These studies have also been used to obtain tolerant plants that can grow on Cd contaminated soils with large amounts of Cd. However, the reasons responsible for such variations in shoot Cd concentration are insufficiently understood.

Padeken (1998) reported that plants grown in Cd contaminated soil with total content of 10 mg kg⁻¹ differed in their shoot Cd concentration. Maize with 0.62 mg Cd kg⁻¹ and sunflower with 1.99 mg Cd kg⁻¹ had low shoot Cd concentration while

flax with $4.87 \text{ mg Cd kg}^{-1}$ and spinach with $6.22 \text{ mg Cd kg}^{-1}$ had high shoot Cd concentration among 13 species tested. The high Cd accumulation was not related to a high root / shoot ratio because sunflower had a ratio of $133 \text{ cm}^2 \text{ g}^{-1}$ and maize of $117 \text{ cm}^2 \text{ g}^{-1}$ while spinach with the higher shoot concentration had a root / shoot ratio of only $28 \text{ cm}^2 \text{ g}^{-1}$ and flax with $87 \text{ cm}^2 \text{ g}^{-1}$ had a middle value. Therefore, spinach had a higher Cd uptake per unit root surface area (269 ng cm^{-2}) than maize (17 ng cm^{-2}). This result may indicate that spinach roots may increase the Cd concentration in the rhizosphere or change the Cd chemistry in the rhizosphere leading to that higher Cd uptake per unit of root. Besides the Cd availability in soil the Cd influx depends also on the root absorbing power, α , defined by the relationship of the net Cd influx, I_n , and the Cd concentration in the solution at the root surface, C_{LO} , ($I_n = \alpha \times C_{LO}$). Plants may differ in their root absorbing power. A variation of α values could be significant for Cd uptake from soil. This aspect of Cd uptake from soil has not been considered. Besides the Cd influx, Cd uptake also depends on the size of the root system. Furthermore, in contrast to essential heavy metal, like Mn, Zn, for non-essential heavy metals as Cd, a large part of the total Cd absorbed is retained in the root and only a smaller portion is transferred to the shoot (Guo et al., 1995; Padeken, 1998; Grant et al., 1998; Keller, 2000; Egle, 2002). A difference in retention of Cd in the roots may be a reason for different Cd concentrations in the shoot. A lower Cd concentration in the shoot could furthermore be explained through a high dilution effect due to a higher relative growth rate. However, little efforts have been made to study simultaneously the possible mechanisms causing the differences in Cd uptake.

The objective was to study to which extent differences in Cd accumulation of different plant species are due to the size of root system, the Cd influx, the growth

rate of the shoot and to the proportion of the Cd taken up that is translocated to the shoot. It was also investigated the effect of plants on Cd availability or solubility in soil and its relation to Cd uptake by the plants. For this, maize, sunflower, flax, and spinach were grown on a soil with three levels of Cd addition and shoot growth, Cd concentration, and root growth were determined. Two harvests were performed in order to be able to measure rates of growth and of Cd uptake.

2.2 Materials und Methods

A soil culture pot experiment was conducted in a climate chamber under controlled conditions (Temperature day/night 25°C / 18 °C, relative humidity day/night: 31% / 60%, light intensity: day/night 16h / 8h, PAR 240 $\mu\text{mol m}^{-2} \text{s}^{-1}$) using four plants species (*Zea mays* L., cv. Cascadas, *Helianthus annuus* L., cv. Ikarus, *Linum usitatissimum* L.ssp. *usitatissimum*, cv. Gold Merchant, *Spinacia oleracea* L., cv. Monnopa) and three Cd additions to the soil (0, 14, and 40 $\mu\text{mol kg}^{-1}$ soil).

The soil used was a sandy soil ($\text{pH}_{\text{CaCl}_2}$ 4.5, 2.8%C, 5% clay). Pots were filled with 3 kg soil and for each Cd addition, one pot was kept unplanted as a control. After Cd addition and basic fertilization of 80 mg N kg^{-1} as $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 100 mg P kg^{-1} as $\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, 80 mg K kg^{-1} as K_2SO_4 , and 10 mg Mg kg^{-1} as $\text{MgSO}_4 \cdot 7 \text{H}_2\text{O}$, soil was incubated at 15% moisture (w/w) for 1 week until Cd concentration in soil solution had stabilized. Then maize, sunflower, flax, and spinach were sown into the pots. The soil was kept at 15% moisture by daily watering of the pots. Plants were harvested after 28 days (harvest I) and after 43 days (harvest II) after sowing. Two harvests were necessary in order to be able to calculate root and shoot growth rates and Cd influx.

Cadmium additions resulted in Cd soil solution concentrations of 0.04, 0.68 and 2.5 $\mu\text{mol L}^{-1}$. The soil solution was attained through a modified displacement technique after Adams (1974). For this, moist soil was taken from unplanted and planted pots. The soil was sieved and about 300g were transferred into 250 mL cylinder, with a drain hole at the bottom covered with a filter paper. At the top of the soil column a 4% KCNS solution was added drop by drop with a rate of about 5 mL h⁻¹ by a peristaltic pump. The KCNS is used to determine whether the displaced solution is the pure soil solution or if it is contaminated. A possible contamination of the displaced solution is detected by adding a few drops of 5% FeCl₃ that in the presence of KCNS produces an intensive red color. Filtrates of soil solution were analyzed for Cd concentration using graphite furnace atomic absorption spectrometer GFAAS. The limits of detection were 2 $\mu\text{g L}^{-1}$.

At each harvest, shoots were cut at the soil level and dried at 105°C. The dried samples were weighed, ground in a mill, and digested in HNO₃ under pressure at 180°C. Digests were analyzed for Cd concentration using flame atomic absorption spectroscopy FAAS. The limits of detection were 2 mg L⁻¹.

The roots were separated from soil by washing and flooding over sieves, and then cleaned of any foreign material. The surface water was removed by keeping them between two filter papers. The fresh root weight was recorded and 0.8 g samples were preserved in 20% ethanol for later measurement of the root length by the line intersect method of *Tennant* (1975).

The net influx (I_n) was calculated using the following equation (Williams 1948)

$$I_n = \frac{(U_{II} - U_I) \cdot \ln\left(\frac{RA_{II}}{RA_I}\right)}{(t_{II} - t_I) \cdot (RA_{II} - RA_I)} \quad \text{Eq. 2-1}$$

where U is shoot Cd content in $\mu\text{mol plant}^{-1}$, RA is root-surface area per plant in cm^2 , t is time of harvest in s, and subscripts I and II refer to first and second harvest, respectively. Assuming exponential growth, the relative growth rate of the shoot (RGR_S) was calculated:

$$RGR_S = \frac{\ln\left(\frac{SDW_{II}}{SDW_I}\right)}{t_{II} - t_I} \quad \text{Eq. 2-2}$$

where SDW is the shoot dry weight and t_I and t_{II} are time of the first and second harvest, respectively.

Assuming a specific gravity for roots of 1 g cm^{-3} , mean root radius (r_0) was calculated using the following equation:

$$r_0 = \sqrt{\frac{RFW}{\pi \cdot RL}} \quad \text{Eq. 2-3}$$

where RFW is the root fresh weight in g and RL is root length in cm.

Mean half distance among neighboring roots (r_1) was calculated from the formula:

$$r_1 = \sqrt{\frac{V_S}{\pi \cdot RL}} \quad \text{Eq. 2-4}$$

where V_S is soil volume in cm^3 and RL is root length in cm.

Factors determining the shoot Cd concentration (X) of plants. Making the simplification that over time X does not change much, it can be shown (Ney and Tinker 1977) that X depends on root surface area (RA) per unit of shoot weight (SDW), the relative shoot growth rate (RGR_S), the Cd total net influx (I_{nt}) and the proportion of absorbed Cd that is translocated from the root to the shoot (p).

$$\text{Shoot Cd concentration } (X) = \frac{RA}{SDW} \cdot \frac{1}{RGR_S} \cdot I_{nt} \cdot p \quad \text{Eq. 2-5}$$

Often only uptake into the shoot is determined and the influx may be named as net shoot influx (In_s), notice that $In_s = In_t \times p$

For each treatment, five seedlings were grown in pots with four replicates per treatment for each harvest in a completely randomized design. The ANOVA was run with the statistic program SigmaStat 5.0.

2.3 Results

The toxic symptoms found included interveinal chlorosis of the leaf (spinach), necrotic leaf spots on the leaves and leaf fall (flax) when exposed to high Cd stress. Roots were brown at high Cd addition (the roots were slightly blackened at their tips). Figure 2-1 shows the effect of Cd addition to the soil on the shoot dry weight (SDW) expressed relative to the un-contaminated soil. Relative SDW were chosen for a better comparison among species. It can be seen that at the low Cd addition of $14 \mu\text{mol kg}^{-1}$ there was small growth stimulation for three species, which was even significant for flax. At $40 \mu\text{mol Cd kg}^{-1}$ soil large differences among species in relative SDW appeared. Maize and sunflower showed little to no yield depression, while the yield of flax and spinach was decreased 20% and 40%

respectively. Flax and spinach were therefore the more sensitive species to Cd toxicity in soil.

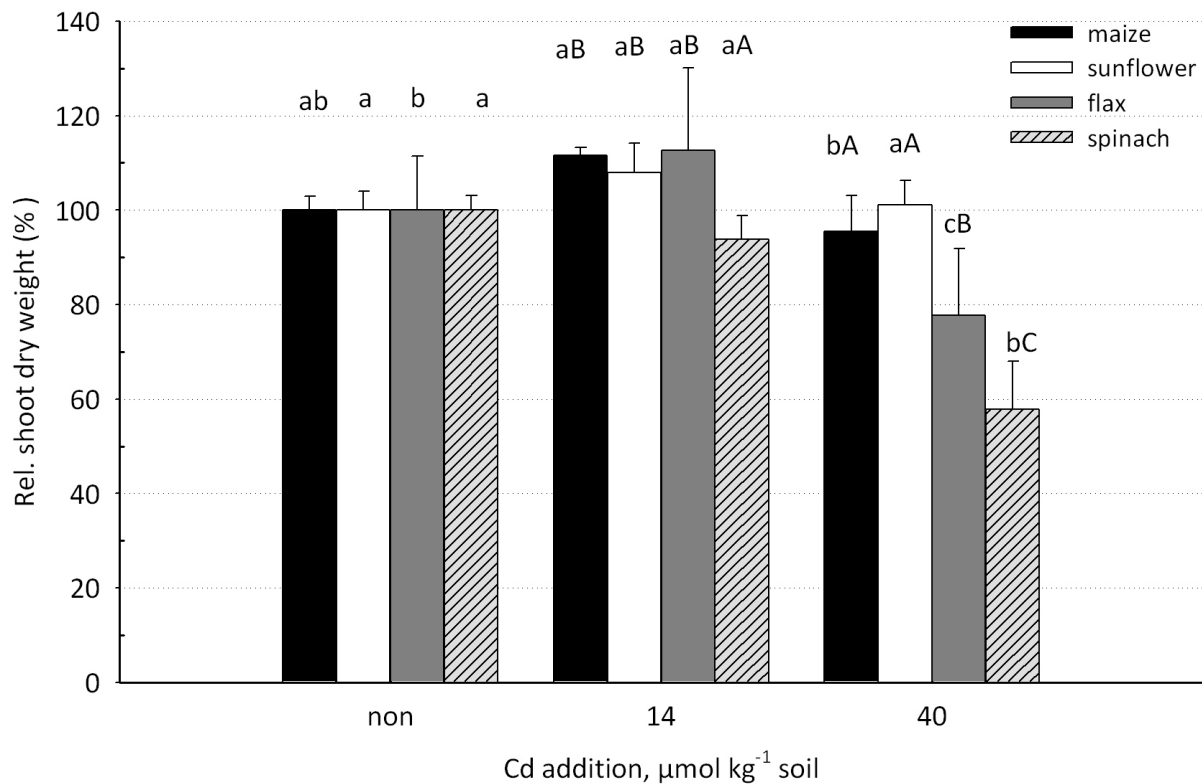


Figure 2-1 Relative shoot dry weight of plants after 43 d growing at different Cd addition to the soil (100% were 7.1 g plant⁻¹ for maize, 3.4 g plant⁻¹ for sunflower, 2.2 g plant⁻¹ for flax and 4.1 g plant⁻¹ for spinach). Different upper case letters show significant differences among plants at the same Cd addition. Different lower case letters show significant differences of the same plant at different Cd additions ($p \leq 0.05$; Student-Newmann-Keuls method).

Table 2-1 shows the accumulation of Cd in shoots and roots of the four species studied. The Cd concentration in shoots and roots were in general similar at first and second harvest. For the same species, the Cd concentration in shoot, and similarly in roots increased about linearly with the Cd addition to the soil without any sign of saturation. Large differences of Cd concentration in shoot, as well as in roots were found among species.

Table 2-1 Root and shoot Cd concentration and content after 28 d and 43 d of growth at different Cd addition to the soil. Translocation to shoot (p) is shoot Cd content expressed as % of total plant Cd. Different upper case letters show significant differences among plants at the same Cd addition. Different lower case letters show significant differences of the same plant at different Cd additions ($p \leq 0.05$; Student-Newmann-Keuls method, n. d.: not determined).

Plant	Cd addition $\mu\text{mol kg}^{-1}$	Shoot Cd concentration $\mu\text{g g}^{-1}$	Root Cd concentration $\mu\text{g g}^{-1}$	Shoot Cd content $\mu\text{g pl}^{-1}$	Root Cd content $\mu\text{g pl}^{-1}$	Traslocation (p) %
Harvest I: 28 days after germination						
Maize	non	0.1aA	n. d.	0.2 aA	n. d.	n. d.
Sunflower	non	0.7 aB	0.9 aA	0.4 aB	0.2 aA	74 aA
Flax	non	1.4 aB	0.6 aA	1.0 aC	0.1 aA	88 bC
Spinach	non	1.3 aB	1.1 aA	1.4 aD	0.4 aB	80 aB
Maize	14	2.4 bA	n. d.	4.3 bA	n. d.	n. d.
Sunflower	14	13.9 bB	16.9 bA	9.7 bB	4.4 bA	69 aA
Flax	14	26.1 bC	18.4 bAB	16.1 bC	3.8 bA	81 aB
Spinach	14	33.6 bD	24.3 bB	36.7 bD	5.8 bB	86 bB
Maize	40	14.3 cA	n. d.	20.2 cA	n. d.	n. d.
Sunflower	40	30.4 cB	44.1 cA	20.4 cA	8.8 cAB	70 aA
Flax	40	71.4 cC	74.4 cB	32.4 cB	9.6 cBC	81 aB
Spinach	40	106.3 cD	79.5 cB	62.8 cC	7.8 bA	89 bC
Harvest II: 43 days after germination						
Maize	non	0.06 aA	0.35 aA	0.4 aA	0.8 aB	35 aA
Sunflower	non	0.4 aB	0.5 aB	1.2 aB	0.7 aB	65 bB
Flax	non	0.9 aB	0.3 aA	2.0 aC	0.3 aA	87 cC
Spinach	non	0.8 aB	1.5 aC	3.4 aD	1.6 aC	68 aB
Maize	14	1.2 bA	2.7 bA	10.0 bA	6.3 bA	60 bA
Sunflower	14	9.7 bB	14.2 bB	35.8 bB	15.2 bB	70 cB
Flax	14	24.6 bC	16.5 bB	61.2 bC	16.8 bC	78 bC
Spinach	14	34.4 bD	21.5 bC	132.2 bD	25.5 bD	84 bD
Maize	40	8.0 cA	12.6 cA	54.3 cA	26.8 cA	67 cB
Sunflower	40	25.8 cB	48.2 cB	88.1 cB	61.9 cC	59 aA
Flax	40	66.4 cC	98.3 cC	113.4 cC	56.2 cC	67 aB
Spinach	40	105.1 cD	93.5 cC	247.0 cD	43.0 cB	85 bC

For example at the second harvest at the lower Cd addition, the shoot Cd concentration was $1.2 \mu\text{g g}^{-1}$ for maize and $34.4 \mu\text{g g}^{-1}$ for spinach and at the high Cd addition, the values were $8 \mu\text{g g}^{-1}$ and $105 \mu\text{g g}^{-1}$ respectively. Spinach accumulated 13 to 30 times more Cd in the shoot than maize. Sunflower and flax were in between.

The differences in root Cd concentration behaved similarly but the differences were not so large. Concerning the distribution of Cd within the plant, i.e. between shoot and root, it can be seen that for maize and sunflower, the concentration in the root is up to two times higher than in the shoot. In contrast, for flax and spinach the Cd concentration is higher in the shoots than in the roots at low Cd addition but at high Cd addition for flax the Cd concentration of the roots became higher than in the shoots and for spinach shoot and root Cd concentration were similar. Cadmium translocation to the shoot varied from 60% to 85%, spinach showing the highest value. This shows that spinach has a lower capacity to retain Cd in the roots, i.e. only 15% compared to 40% of maize.

Table 2-2 shows the effect of Cd on root growth of the four plants species. The root length density (RL_V) characterizes the size of the root system since all plants grew in the same volume of soil. The RL_V allows also calculating the half distance between neighboring roots (r_1). It can be seen that maize and sunflower had larger root systems than flax and spinach at all Cd levels. The addition of Cd of $14 \mu\text{mol kg}^{-1}$ had a stimulating effect on root growth except in sunflower where RL_V stayed constant. At high Cd addition, this stimulating effect was still there for maize but in spinach, a strong decrease of almost 50% occurred. The half distance between neighboring roots ranged mostly from 0.1 cm to 0.2 cm except at high Cd addition it was 0.3 cm for spinach. This shows that the whole soil was close to a

root so that the activity of the root could have influenced the properties of the whole soil volume. The root radius (r_0) differed somewhat among species and decreased with Cd addition around 5% to 10%.

Table 2-2 Root length density (RL_V), root radius (r_0) and half distance between neighboring roots (r_1) at different Cd addition to the soil at second harvest (43d after germination). Different upper case letters show significant differences among plants at the same Cd addition. Different lower case letters show significant differences of the same plant at different Cd additions ($p \leq 0.05$; Student-Newmann-Keuls method).

Plant	Cd addition $\mu\text{mol kg}^{-1}$	RL_V cm cm^{-3}	r_1 cm	r_0 cm
Maize	non	20 Da	0.13 Ab	0.024 Cb
Sunflower	non	17 Cbc	0.14 Ba	0.018 Ab
Flax	non	9 Ba	0.19 Cb	0.019 Bb
Spinach	non	6 Ab	0.23 Db	0.024 Cb
Maize	14	28 Dc	0.11 Aa	0.020 Ca
Sunflower	14	16 Cab	0.14 Ba	0.017 Aa
Flax	14	13 Bb	0.16 Ca	0.019 Bb
Spinach	14	8 Ac	0.20 Da	0.022 Da
Maize	40	25 Db	0.11 Aa	0.021 Ca
Sunflower	40	18 Cc	0.14 Ba	0.017 Aa
Flax	40	8 Ba	0.21 Cc	0.018 Ba
Spinach	40	3 Aa	0.32 Dc	0.023 Da

The Cd influx into roots can be estimated by the increase of Cd content of the plants (shoot plus roots) with time divided by the root surface area and the time

of uptake. In soil-grown plants, the Cd content of the roots cannot always be determined reliably because of their possible contamination with Cd-rich soil particles. Therefore in this paper to calculate the Cd influx only the increase of Cd content of the shoots was used and the influx is named “shoot influx”. The results in Fig. 2-2 show that the Cd shoot influx for sunflower and flax increased linearly with the Cd addition but for spinach and maize, the increase was more than proportional. For a 3-fold increase of Cd addition (14 to $40 \mu\text{mol kg}^{-1}$) the Cd influx increased 8-fold (from 0.2×10^{-16} to $1.6 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$) for maize and 6-fold (from 7.5×10^{-16} to $42 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$) for spinach.

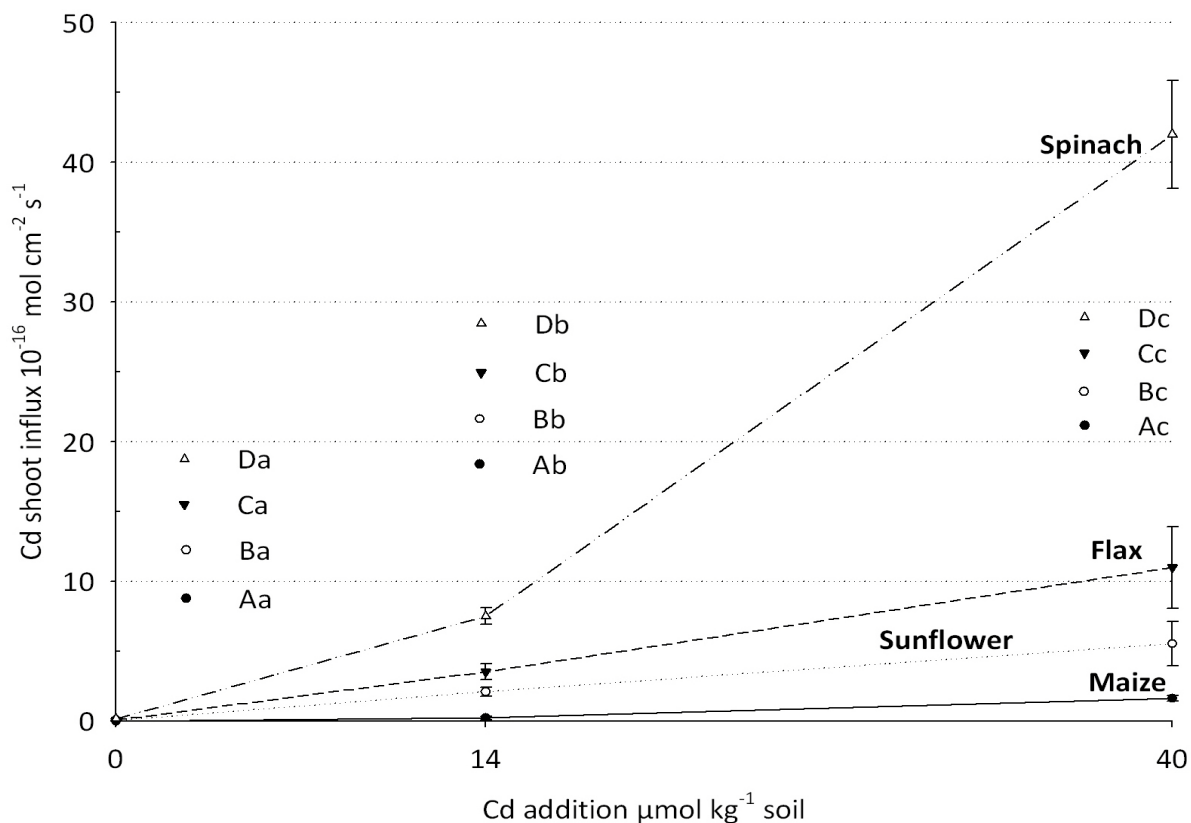


Figure 2-2 Shoot Cd influx at different Cd addition to the soil. Different upper case letters show significant differences among plants at the same Cd addition. Different lower case letters show significant differences of the same plant at different Cd additions ($p \leq 0.05$; Student-Newmann-Keuls method).

At all Cd levels, the influx differed significantly among all species, maize always having the lowest and spinach the highest Cd influx. The Cd influx of spinach was around 30 times higher than that of maize. As already seen in Table 2.1 the shoot Cd concentration differed among species by a factor of up to 30. Table 2-3 summaries the factors that according to equation 2-5 would be responsible for those differences.

Table 2-3 Plant factors determining the shoot Cd concentration; root area / shoot dry weight ratio (RA / SDW), shoot growth rate (RGR_s) and shoot influx at different Cd addition to the soil after 43 d of growth. Different upper case letters show significant differences among plants at the same Cd addition. Different lower case letters show significant differences of the same plant at different Cd additions ($p \leq 0.05$; Student-Newmann-Keuls method).

Plant	Cd add $\mu\text{mol kg}^{-1}$	shoot Cd mg kg^{-1}	RA / SDW $\text{cm}^2 \text{g}^{-1}$	RGR _s 10^{-6}s^{-1}	Shoot Influx $10^{-16} \text{mol cm}^{-2} \text{s}^{-1}$
Maize	non	0.06 aA	408 aB	1.30 aB	0.01 aA
Sunflower	non	0.4 aB	534 bD	1.40 aB	0.06 aB
Flax	non	0.9 aB	463 aC	0.81 aA	0.09 aC
Spinach	non	0.8 aB	236 aA	0.90 aA	0.17 aD
Maize	14	1.2 bA	433 abB	1.34 aB	0.2 bA
Sunflower	14	9.7 bB	437 aB	1.37 aB	2.1 bB
Flax	14	24.6 bC	588 cC	1.01 bA	3.5 bC
Spinach	14	34.4 bD	305 bA	0.86 aA	7.5 bD
Maize	40	8.0 cA	475 bBC	1.40 aB	1.6 cA
Sunflower	40	25.8 cB	543 bD	1.35 aB	5.6 cB
Flax	40	66.4 cC	521 bCD	0.96 bA	11.0 cC
Spinach	40	105.1 cD	197 aA	0.94 aA	42.0 cD

It can be seen that for the root-shoot ratio the highest and lowest value differed by a factor of two to three, spinach having the lowest value which would rather cause a lower Cd shoot concentration in contrast to what actually was found. The relative shoot growth rate (RGR_S) varied by a factor of maximum of 1.7. In contrast, the Cd shoot influx varied by a factor of up to 37. It was mostly the Cd influx that determined the different Cd concentration in the shoot. Spinach had the highest Cd influx and the highest Cd shoot concentration and maize had the lowest Cd influx and the lowest shoot Cd concentration.

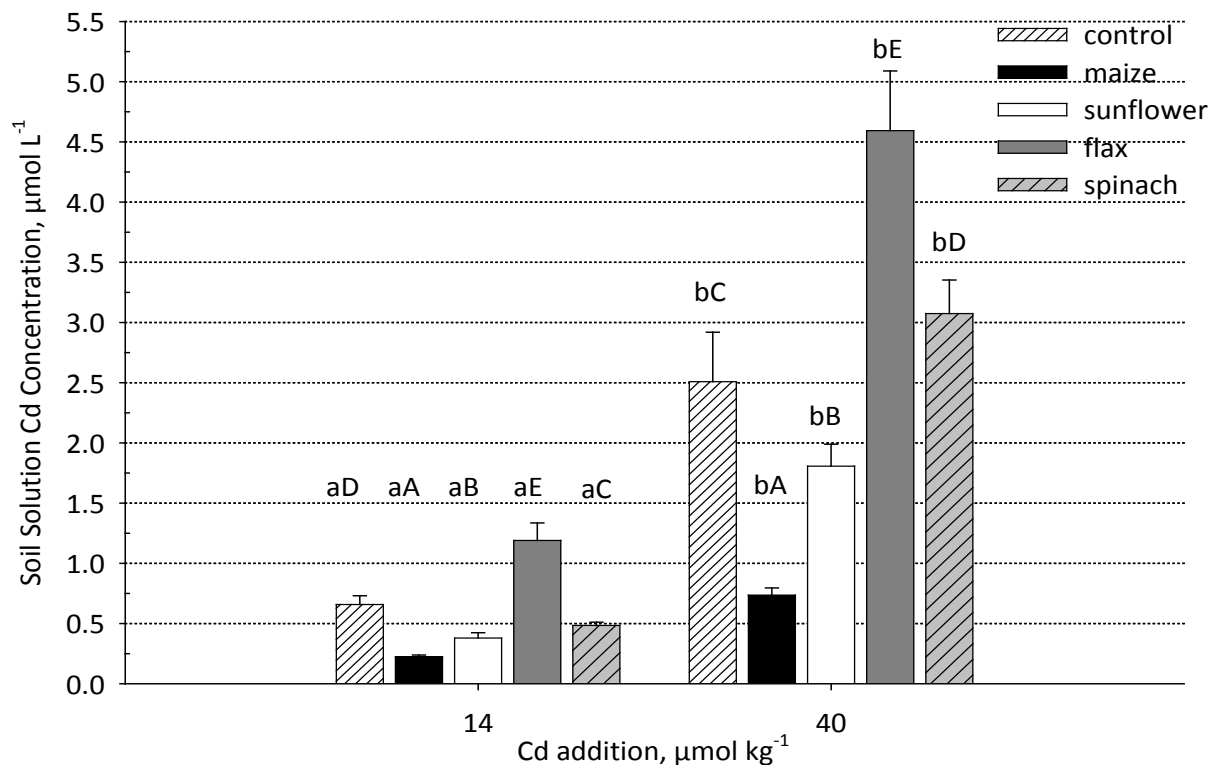


Figure 2-3 Cadmium in soil solution at different Cd addition to the soil (average between 1st and 2nd harvest). Different upper case letters show significant differences among plants at the same Cd addition. Different lower case letters show significant differences of the same plant at different Cd additions ($p \leq 0.05$; Student-Newmann-Keuls method).

In order to investigate whether the activity of the roots changed the Cd availability in soil that might explain the different Cd influx shown before. The Cd concentration in soil solution after different plants had grown in the soil is shown in Fig. 2-3. The soil solution concentration varied somewhat from first to second harvest, either decreasing or also increasing (see Tab. 8-3 Appendix), therefore the average Cd concentration of the two harvests is shown in Fig. 2-3 and Fig. 2-4. The concentrations of Cd in the unplanted soil (control) increased almost linearly with increasing Cd addition. Plant growth changed Cd concentration in soil solution. The Cd concentration in soil solution significantly decreased after the growth of maize, sunflower, and spinach at the low Cd addition. For example, Cd concentration was decreased by about 65% after the growth of maize and by about 41% after the growth of sunflower compared to the control. The soil solution concentration of Cd was significantly higher (84%) than the control after the growth of flax in the soil of $14 \mu\text{mol Cd kg}^{-1}$. At the high Cd addition ($40 \mu\text{mol Cd kg}^{-1}$) also spinach increased Cd soil solution concentration but much less than flax.

To investigate whether these changes in Cd solution concentration may explain the different Cd influx, the shoot Cd influx was plotted against average Cd concentration in soil solution between first and second harvest (Fig. 2-4). For all plants the relationship was about linear. It can be seen that maize, sunflower and flax adjusted to about the same line (slope around $2.5 \times 10^{-7} \text{ cm s}^{-1}$) while spinach showed a clearly, 5 times, higher slope of $13.6 \times 10^{-7} \text{ cm s}^{-1}$. For maize and sunflower, the Cd concentration range was up to $2 \mu\text{M Cd}$ while for flax and spinach it reached $5 \mu\text{M Cd}$ in the soil solution (see inset).

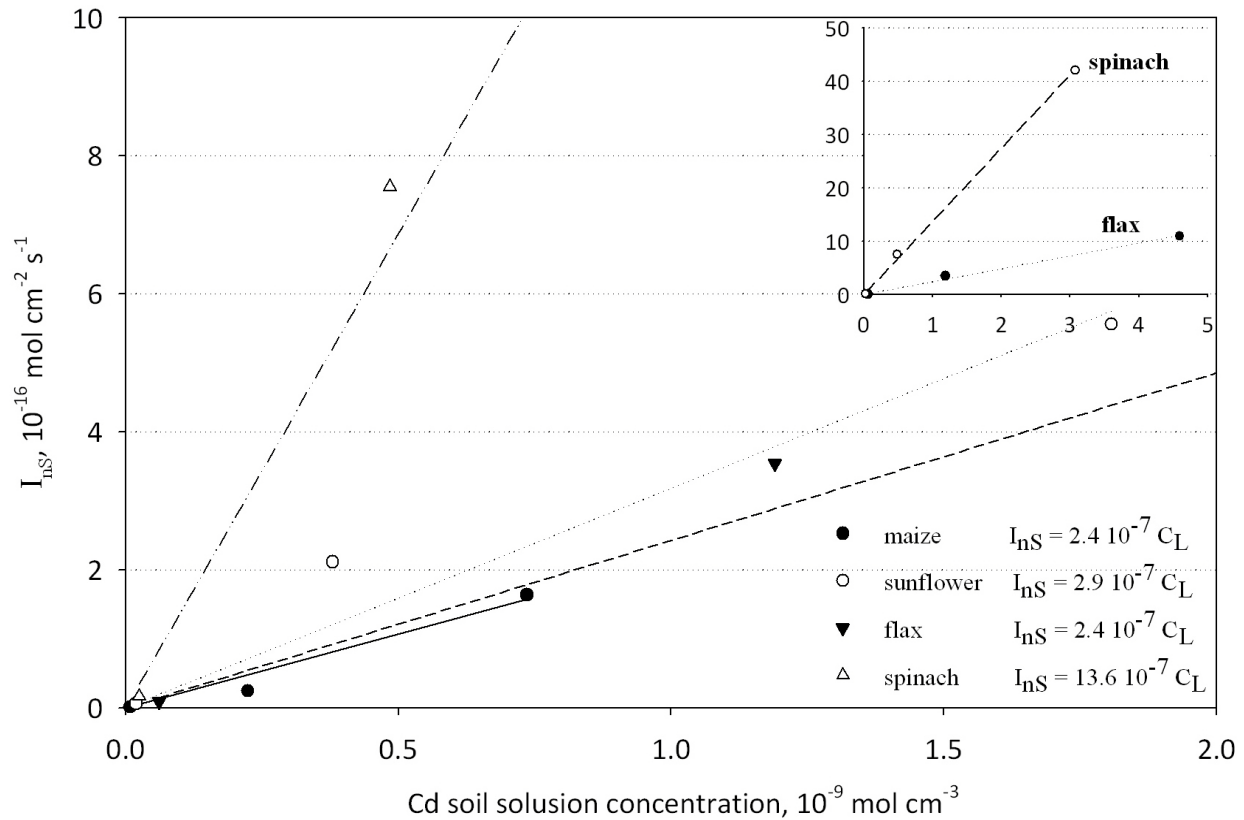


Figure 2-4 Shoot Cd influx against average Cd concentration in soil solution between first and second harvest after Cd addition.

2.4 Discussion

In the current experiment, we compared Cd accumulation and sensitivity or tolerance of the four plant species, maize (*Gramineae*), sunflower (*Asteraceae*), flax (*Linaceae*) and spinach (*Chenopodiaceae*) in order to find out possible factors and processes responsible for differences in shoot Cd accumulation when grown in the same soil.

The Cd concentration in soil solution without Cd contamination was 0.04 μM , which is low and found as tolerable for healthy growth of plants. The addition of 14 $\mu\text{mol kg}^{-1}$ Cd to the soil did not inhibit, but slightly stimulated plant growth. Wu

et al., 2003, Dong et al., 2005, found similar results. Adding 40 $\mu\text{mol Cd kg}^{-1}$ to the soil caused an inhibition of growth in flax and spinach only, indicating that they are Cd sensitive while the Cd tolerant plants (maize and sunflower) had a SDW of almost 100%. Kloke and Schenke (1979) classified spinach also as Cd sensitive. The sensitivity of flax and spinach to soil Cd was confirmed by severe Cd toxicity symptoms. Similar toxicity symptoms reported Dong et al. (2005), in tomato, Salt et al. (1995) in mustard. The symptoms may be related to that Cd exerts a direct toxic effect, perhaps by affecting the process of photosynthesis or the activity of various enzymes (Weigel et al., 1980; Uraguchi et al., 2006), by reducing the chlorophyll content and/or transpiration (Haag-Kerwer et al., 1999; Vassilev et al. 2002; Hassan et al., 2005). The addition of 40 $\mu\text{mol Cd kg}^{-1}$ soil was clearly toxic to flax and spinach but not to maize and sunflower. Different tolerance to soil Cd has often been associated to two different strategies, i.e. exclusion and accumulation (Baker et al 2000, Yang et al., 2004). In our case the different tolerance among species to soil Cd is clearly related to the different Cd accumulation in the shoot.

We used equation 2-5 to quantify the factors responsible for different Cd accumulation in the shoot. The proportion of Cd translocated to shoot (Tab. 2-1) varied in the low (14 $\mu\text{mol Cd kg}^{-1}$) and high (40 $\mu\text{mol Cd kg}^{-1}$) addition from 60% to 70% in the less sensitive species and from 70% to 85% in the more sensitive species. Spinach the most sensitive species always showed the highest translocation rate. However, the variation of 60% to 85% could not explain the variation of shoot Cd concentration by a factor of about 10 to 30. Similarly, the variation of the root-shoot ration and the relative shoot growth rate was low and could not explain the variation in shoot Cd concentration (Tab. 2-3).

Only the Cd net influx varied parallel to the shoot Cd concentration, i.e. the Cd influx was the major factor responsible for the different Cd accumulation in the shoot. Why this differences in Cd influx?

A possible mechanism for different Cd influx among plants may be because plants can affect the Cd availability in soil. Padeken (1998) found that the CaCl_2 extractable soil Cd varied among plants by a factor of two. Instead of using a Cd extraction like Padeken (1998) we used the Cd concentration in soil solution, because Cd influx depends on Cd concentration in soil solution at the root surface and also Cd transport to the root is in the soil solution. In fact, plants had a strong effect on Cd concentration in soil solution, i.e. plant growth decreased as well as increased Cd concentration in soil solution (Fig. 2-3). The decrease of Cd in soil solution at low Cd addition was not related to Cd uptake by the plants, since maize with the lowest amount of Cd removed from soil (about $0.15 \mu\text{mol kg}^{-1}$) showed the strongest decrease of Cd in soil solution. Whereas spinach with the highest amount of Cd removed, (about $1.4 \mu\text{mol kg}^{-1}$) showed the least decrease of Cd in soil solution. This indicates that root activity of maize caused a Cd immobilization in soil. Egle (2002) observed similar immobilization of Cd for several lupine genotypes and for *Lolium multiflorum*. Morel et al. (1986) have shown that mucilage, a high-molecular weight exudate, of maize rhizosphere are composed mainly of polysaccharidic and contain proteins (4%) and a high proportion of uronic acids (34%) was able to bind Cd. This high binding ability of mucilage of maize could explain the Cd immobilization in soil. Furthermore, it might be stressed that the composition of root exudates probably depends on the nutrient status of the plants and Cd stress condition (Keller, 2000; Egle, 2002).

In contrast to decreasing Cd soil solution concentration flax at both Cd levels and spinach at the high Cd level increased Cd soil solution concentration. This situation can occur when plants solubilize more Cd as is taken up by them. The Cd solubility in soil may be affected by chemical action of root exudates or microbial activity in the rhizosphere that increase Cd concentration in soil solution (0.68 μ M to 1.2 μ M). The differences in Cd concentration in soil solution could be the result of the plants response to varying soil Cd levels possibly affecting soil particle surface. Cieslinski et al. (1998) reported differences in total low-molecular-weight organic acids detected in the rhizosphere soil of *durum* wheat species that was related to NH₄Cl extractable Cd indicating differences in rhizosphere dynamics, uptake and Cd distribution within plants. Furthermore, plant roots contain many organic anions varying in chain length with lactate, acetate, oxalate, succinate, fumarate, malate, citrate, isocitrate, and aconitate being the main components and potential metal chelators (Jones, 1998; Marschner, 1995). These differences in composition of exudates could influence the rhizosphere, with both an increase or decrease in the Cd activity may be present in the rhizosphere (Mench and Martin, 1991; Egle 2002).

In order to test whether the changes of Cd concentration in soil solution can explain the different Cd influx among plants we plotted the Cd influx against average Cd concentration in soil solution after growing the plants (Fig. 2-4). Comparing maize, sunflower and flax the changes of Cd soil solution concentration could to a large extent explain the differences of Cd influx observed, i.e. they laid on about the same line and if flax had a larger Cd influx than sunflower and maize it was because it increased C_{Li} the strongest. In contrast, spinach differs from these three plant species in that at the same Cd concentration in soil solution it

had a Cd influx about 5 times higher. This different behavior of spinach may be related to spinach having a higher root absorbing power, i.e. at the same concentration at the root surface, Cd influx is higher. To our knowledge, there are no data of root absorbing power of plants in the concentration range we found in soil solution. It also may be related to that in the case of the other plants (maize, sunflower and flax) part of the Cd in soil solution may be present as chelates. Chelated ions are usually not available for adsorption by roots (Senden et al., 1995). This agrees with the findings of Cabrera et al. (1988) who found that humic acid lowered Cd uptake of barley plants (*Gramineae*) grown in hydroponic solutions containing Cd concentrations (0.5-5 mg L⁻¹) and humic acid (190-1710 mg L⁻¹).

In summary, the results show that shoot Cd concentration was very different among the plant species which was mainly related to the Cd influx, which in the same soil differed by a factor of 30 while the size root system and shoot growth rate had a little effect. Plants affected Cd availability in soil, in some cases decreasing in other cases increasing C_{Li} i.e. plants immobilized or solubilized Cd. However, these changes in C_{Li} were not enough to explain differences in Cd influx.

We hypothesize that plant species furthermore differ in their root absorbing power and that possibly part of Cd in soil solution is present as a complex, which is not available for uptake by the roots. In order to validate the findings in soil experiment will need to be investigated further by comparing the results of an experiment with nutrient solution (Stritsis 2005), in which he had found the relationship between the Cd influx and Cd concentration at the root surface (i.e. root absorbing power). The comparison will be performed in the next chapter

using a mechanistic model (NST 3.0) of Cd uptake from the soil to find further explanations for the differences in Cd influx.

3 MECHANISTIC SIMULATION AND MEASURED CADMIUM UPTAKE FROM SOIL

3.1 Introduction

Cadmium uptake and Cd shoot concentration differed strongly among species. These differences were mainly due to different influx that varied among them by a factor of up to 30, even though the plants had been grown in the same soil (Chap. 2). The Cd influx depends on Cd transport in the soil and on uptake physiology of the plants. It could be shown that the Cd concentration in soil solution, C_{Li} , as important parameter of Cd transport in the soil and of uptake into the plant was different among plant species (Chap. 2). However, this did not totally explain the observed differences in their Cd influx. In this chapter, further explanations will be sought by using mechanistic modeling of Cd uptake from soil as will be explained below.

For the last 50 years, the processes governing the ion transfer from soil into the root have been investigated. They are the transport from soil to the root surface by mass flow (F_M) and diffusion (F_D) (Barber, 1962) and the uptake into the root, which depends on the ion concentration in the soil solution at the root surface. The latter usually follows a saturation curve that can be described by a Michaelis-Menten function (Epstein, 1972). The transport by mass flow depends on the concentration in soil solution and the water flow towards the root. Diffusion depends on soil parameters like moisture content and buffer power, but is driven by the concentration gradient from bulk soil to root surface. This gradient is

established by plant uptake and depends therefore, on uptake kinetics. Hence, ion uptake from soil is the result of an interaction of soil and plant processes and factors. Mathematical models have been used to simulate these interacting processes (Nye and Marriott, 1969; Claassen and Barber, 1976; Barber and Cushman, 1981; Claassen and Steingrobe, 1999). These models calculate an ion uptake based on soil and plant parameters, which are independent of the measured uptake itself. The models have mainly been used to describe P or K uptake from soil. In some cases, these models calculated similar results as observed, for example, Seiffert et al. (1995) clearly described the K uptake of maize at different soil bulk density and water content. Such results show that the models are able to describe soil transport and uptake. However, simulated P uptake of wheat and sugar beet growing in low P soils was often underestimated (Bhadoria et al., 2002). This shows that uptake conditions might be more complex than can be described by mass flow, diffusion, and Michaelis-Menten kinetics or that the estimation of soil and plant parameters was not adequate. A sensitivity analysis for example indicated that most likely plants may have increased P soil solution concentration, i.e. they mobilized soil P, whereas changes of uptake kinetic parameters were of low effect (Bhadoria et al., 2002).

The objective of this research was to use a mechanistic model to describe the Cd influx of maize, sunflower, flax and spinach and to use a sensitivity analysis not only on soil but also on plant parameters in order to find possible reasons for differences of Cd influx observed among plant species.

3.2 Materials und Methods

The model NST 3.0 (Claassen 1990; Claassen and Steingrobe, 1999) calculates soil transport of nutrients towards the root by diffusion and mass flow taking sorption processes to the soil matrix into account. The uptake into the root is assumed to follow Michaelis-Menten kinetics. The model was run to simulate the Cd uptake from soil described in Chap.2. The soil parameters and some plant parameters were taken from that same experiment and uptake kinetic parameters were from earlier nutrient solution experiment (Stritsis, 2005). Calculations were performed for the time period between day 28 (harvest I) and 43 (harvest II) after germination.

Soil parameters

D_L : Diffusion coefficient in water ($7.2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$) obtained from Parsons (1959)

Θ : Volumetric soil water content, was obtained form gravimetric water content times soil bulk density ($\text{cm}^3 \text{ cm}^{-3}$)

f : The impedance factor which depends on Θ . It was calculated according to Barraclough and Tinker (1981); $f = 1.58 \times \Theta - 0.17$

C_{Li} : Initial Cd soil solution concentration, measured in unplanted and planted pots at day 28 ($\mu\text{mol L}^{-1}$ solution)

b : buffer power, assuming a linear buffer curve, b was calculated form Cd added ($\mu\text{mol L}^{-1}$ soil) to the soil divided by C_L ($\mu\text{mol L}^{-1}$ solution)

Plant parameters

r_0 : root radius, (cm)

r_i : Half-distance among neighboring roots, (cm)

k : Relative root growth rate, (d^{-1})

L_0 : Root length at first harvest, (cm)

Uptake kinetic parameters

The uptake kinetics describes the relationship between the net influx, I_n ($\text{mol cm}^{-2} \text{s}^{-1}$), and the concentration at the root surface, C_{LO} . For Cd in the low concentration range, as used in this study, this relationship is linear (Stritsis, 2005) of the form

$$I_n = \alpha \cdot C_{LO} \quad \text{Eq. 3-1}$$

α (cm s^{-1}) being called the root absorbing power (Nye and Tinker, 1977)

The NST 3.0 simulation model uses the saturation kinetics of Michaelis-Menten

$$I_n = \frac{I_{\max} \cdot C_{LO}}{K_m + C_{LO}} \quad \text{Eq. 3-2}$$

I_{\max} is the maximum influx and K_m is the Michaelis constant, i.e. the concentration at which $I_n = I_{\max} / 2$

In the low concentration range, clearly below K_m , the uptake isotherm can be approximated by a straight line given by

$$I_n = \frac{I_{\max}}{K_m} \cdot C_{LO} \quad \text{Eq. 3-3}$$

that means, α of equation 3-1 is given by I_{\max} / K_m .

In this study α was known from studies in solution culture (Stritsis, 2005) and setting K_m at $10\mu\text{M}$, i.e. clearly above the Cd concentration found in soil solution, I_{\max} for model calculation is given as follows

$$I_{\max} = \alpha \cdot K_m \quad \text{Eq. 3-4}$$

It has to be stated very clearly that I_{\max} and K_m were not measured directly by fitting a Michaelis-Menten curve to the experimental data. The concentration range was very low, i.e. it was in the quasi-linear range of the Michaelis-Menten curve, not allowing a satisfying fitting. Therefore, I_{\max} was derived from the α value under an assumption of a given K_m value. The α values were taken from Stritsis (2005).

3.3 Results

The simulation of the Cd uptake was performed between two harvests, 28 and 43 days after germination. Cadmium uptake kinetics from the nutrient solution experiment together with other soil and plant parameters from the soil experiment were used to simulate Cd uptake from soil. The input parameters used in the model are summarized in Table 3-1 and were taken from experiment of Chap. 2, and the α values from Stritsis (2005).

Soil solution Cd concentration, C_{Li} , was different among the plants even though they grew in the same soil. It varied by a factor of 6 because of the activity of the roots. According to changes of C_{Li} also the buffer power, b , changed. Value of root absorbing power, α , varied by a factor of about 3. Spinach and flax had a similar and higher α value than maize and sunflower. To calculate the total Cd uptake of the growing root system, based on the uptake per cm, the parameter k (relative

growth constants) and L_0 (initial root length at the first harvest) are needed. However, comparison between calculated and measured data will be done on the basis of influx.

Table 3-1 Parameters used for Cd-uptake model calculations

	Plants							
	Maize	Sunflower	Flax	Spinach	Maize	Sunflower	Flax	Spinach
Cd addition	----- 14 $\mu\text{mol Cd kg}^{-1}$ -----				----- 40 $\mu\text{mol Cd kg}^{-1}$ -----			
Parameters								
α value ($10^{-6} \text{ cm s}^{-1}$)	1.2	1.7	2.7	3.2	1.2	1.7	2.7	3.2
I_{max}^s ($10^{-14} \text{ mol cm}^{-2} \text{ s}^{-1}$)	1.2	1.7	2.7	3.2	1.2	1.7	2.7	3.2
v_0 ($10^{-7} \text{ cm}^3 \text{ cm}^{-2} \text{ s}^{-1}$)	5.7	14	10.4	11.2	5.7	13	15.4	23.8
b	84	50	20	58	51	25	16	17
C_{Li} ($10^{-9} \text{ mol cm}^{-3}$)	0.22	0.38	1.2	0.48	0.74	1.8	4.6	3.0
r_0 (cm)	0.024	0.019	0.016	0.018	0.023	0.015	0.016	0.02
r_1 (cm)	0.192	0.228	0.223	0.216	0.197	0.229	0.304	0.426
L_0 (cm)	5215	3763	4008	4227	5028	3702	2264	1087
k (d^{-1})	0.128	0.102	0.072	0.041	0.124	0.111	0.08	0.065

Parameter for all treatments: $D_L = 7.2 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$, $K_m = 1 \times 10^{-8} \text{ mol cm}^{-3}$, $C_{min} = 0 \text{ mol cm}^{-3}$, $\Theta = 0.2 \text{ cm}^3 \text{ cm}^{-3}$,
 $f = 0.15$ ($f = 1.58 \times \Theta - 0.17$), $I_{max}^s = \alpha \times K_m$

Measured and calculated Cd influx is shown in Table 3-2. At $14 \mu\text{mol Cd kg}^{-1}$, measured Cd influx was $0.25 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$ for maize, which was 10-fold lower than that of sunflower and 30-fold lower than that of spinach. Under high Cd addition, Cd influx of maize was 26-fold lower than that of spinach. After calculation with NST 3.0, calculated Cd influx was $2.4 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$ for maize, which was only 2-fold lower than that of sunflower and 5-fold of spinach. Under high Cd addition, Cd influx of spinach was only 10-fold higher than that of maize.

Comparing calculated and measured values, it can be seen that the calculated Cd influx does not agree with measured values. Under low Cd addition Cd influx for maize was overestimated 10 fold and for flax 7 fold whereas for spinach it was only 1.6. Under high Cd addition, the overestimation was lower for maize (4.7) and for sunflower (4.7) and for flax was similar to the low Cd addition. However, prediction was closer for spinach, being 1.6 under lower Cd addition and 1.8 under higher Cd addition of the measured influx.

Table 3-2 Calculated shoot influx in comparison to measured shoot influx of maize, sunflower, flax and spinach at different Cd addition to the soil.

Plant	Influx $10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$		
	measured	calculated	Calc / Meas.
14 $\mu\text{mol Cd kg}^{-1}$			
Maize	0.3	2.4	9.8
Sunflower	2.1	5.3	2.5
Flax	3.5	24.4	6.9
Spinach	7.5	12.0	1.6
40 $\mu\text{mol Cd kg}^{-1}$			
Maize	1.6	7.7	4.7
Sunflower	5.6	25.9	4.7
Flax	11.0	82.2	7.5
Spinach	42.1	75.0	1.8

A sensitivity analysis will help to find possible reasons for the overestimation of Cd influx. In the sensitivity analysis, reduced values of the initial solution Cd concentration and root absorbing power were used. A sensitivity analysis in soil buffer power is not shown because there was almost no effect.

Figure 3-1 shows the influence of the change ratio of C_{Li} on calculated Cd influx at $14 \mu\text{mol Cd kg}^{-1}$. It can be seen that the relationship between C_{Li} and calculated Cd influx was almost linear. The figure also shows the point of the change ratio at which the calculated Cd influx is equal to measured value. For sunflower, the calculated Cd influx was similar to measured Cd influx when C_{Li} was reduced to 35% while for flax the reduction was to 15% (Fig. 3-1). A decrease in C_{Li} to 63% resulted in 100% prediction by the model for spinach. For maize, the sensitivity analysis for C_{Li} showed that the change ration should be close to 10% to get 100% prediction by the model.

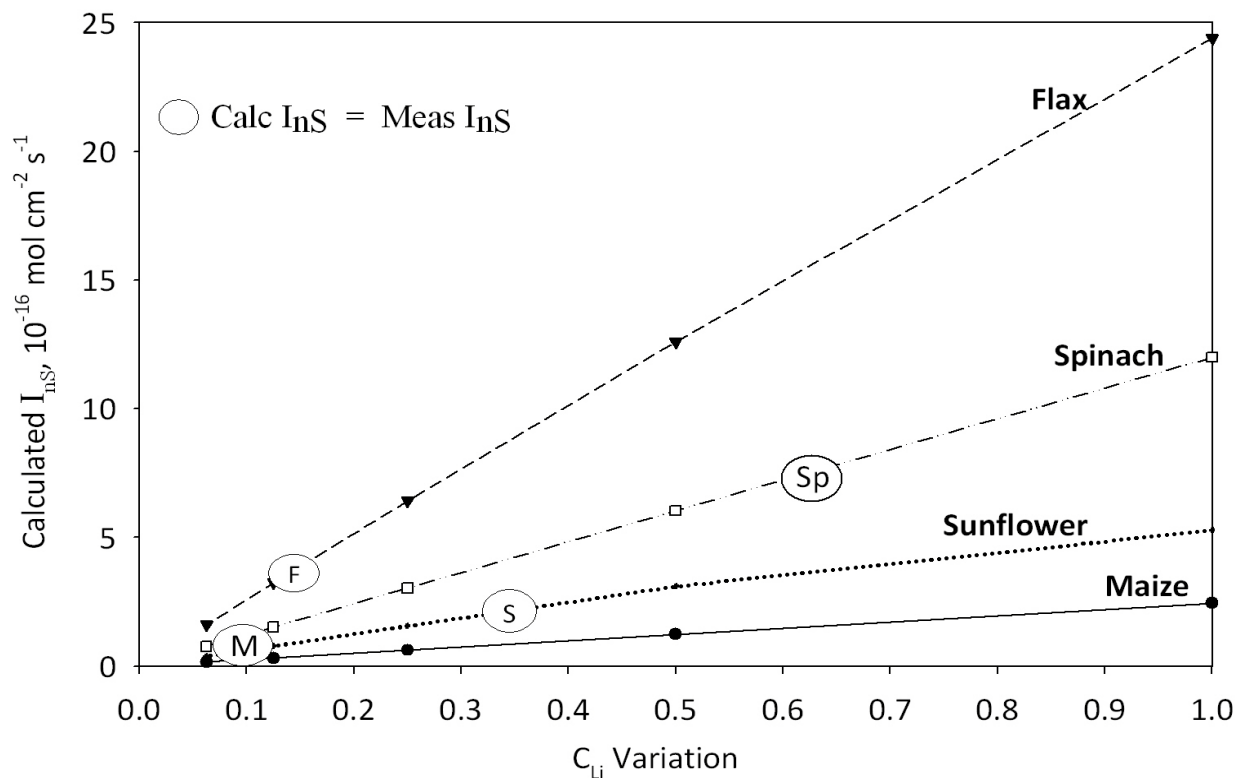


Figure 3-1 Sensitivity analysis. Response of calculated influx to changes in the initial soil solution concentration (C_{Li}). Initial data set of the treatment with an application of $14 \mu\text{mol Cd kg}^{-1}$. M: maize, S: sunflower, F: flax, Sp: spinach. Circle with letter indicates at which change ration calculated influx is equal to measured influx

Table 3-3 summarizes the sensitivity analysis of varying C_{Li} as well as α at both Cd levels for all plants. The values shown in the first line of Table 3-3 are the results shown in Fig. 3-1.

The table gives the change needed of the soil solution concentration (C_{Li}) and the root absorbing power (α) so that the calculated shoot influx is equal to the measured shoot influx. The sensitivity analysis for C_{Li} and α value show that by decreasing their values, change ratio <1 , simulated Cd influx could become 100% prediction of measured Cd influx. A decrease in C_{Li} by 90% (change ratio = 0.1) resulted in 100% prediction by the model for maize at $14 \mu\text{mol Cd kg}^{-1}$ addition in soil while at $40 \mu\text{mol kg}^{-1}$ the decrease needed was 80%. The effect of changing α value was similar to changing C_{Li} .

Table 3-3 The values are the factors (change factor) by which the original Cd soil solution concentration, C_{Li} , or the root absorbing power, α , had to be changed so that the calculated influx became equal to the measured Cd influx.

Cd add $\mu\text{mol kg}^{-1}$	Parameter	Maize	Sunflower	Flax	Spinach
		change ratio			
14	C_{Li}	0.10	0.33	0.15	0.63
14	α	0.10	0.31	0.11	0.54
40	C_{Li}	0.21	0.21	0.11	0.53
40	α	0.21	0.21	0.11	0.49

To get a better understanding of the Cd dynamics in the rhizosphere and to get possible explanation for the results of the sensitivity analysis of C_{Li} and α the calculated concentration profiles around roots were studied.

Some examples are shown in Fig. 3-2 and Fig. 3-3 for the treatment of $14 \mu\text{mol kg}^{-1}$ Cd addition, for one with the original values of C_{Li} and α and for another with

the values for C_{Li} or α where the calculated Cd influx became equal to the measured Cd influx. Figure 3-2A shows for maize that the initial Cd concentration of $0.22 \mu\text{M}$ was decreased at the root surface to only $0.19 \mu\text{M}$ after 14 d of uptake and even so the calculated Cd influx was 10 times higher ($2.4 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$) than the measured Cd influx ($0.25 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$). For spinach (Fig 3-3A), the initial Cd concentration of $0.48 \mu\text{M}$ was decreased at the root surface to $0.36 \mu\text{M}$ after 14 d of uptake. In addition, here the calculated Cd influx was higher but only 1.6 fold higher than the measured Cd influx.

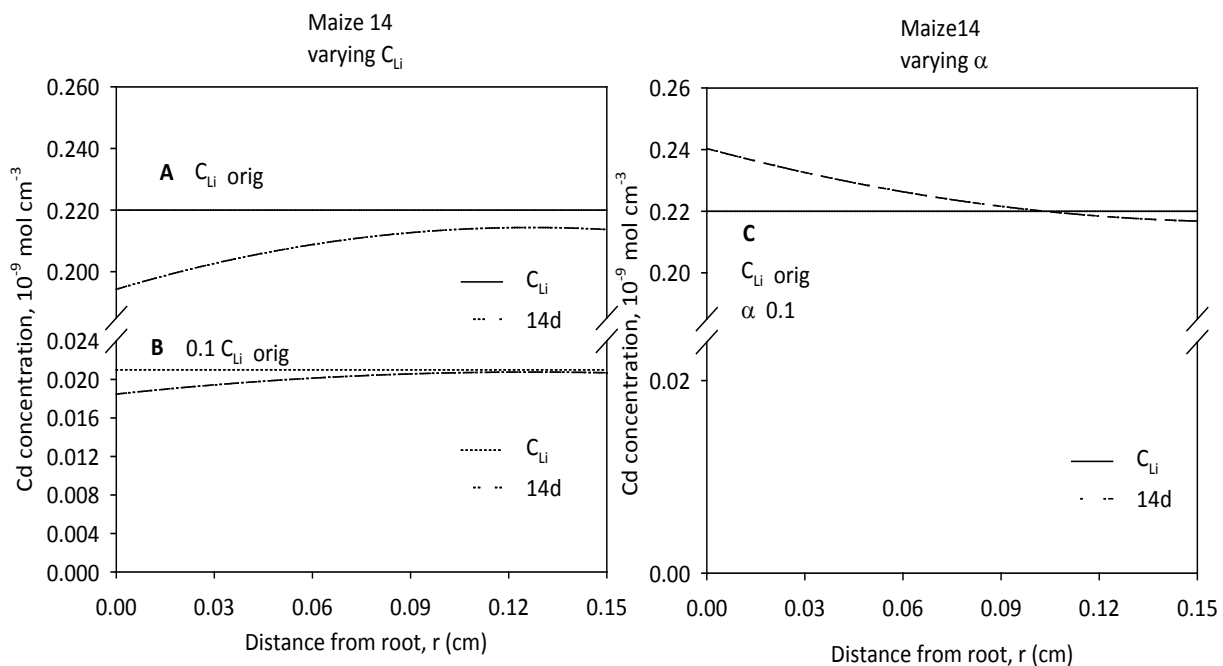


Figure 3-2 Calculated depletion of Cd from the rhizosphere of maize after 14 days of uptake at $14 \mu\text{mol Cd kg}^{-1}$ used NST 3.0 (A). Effect of changing C_{Li} or α value on calculated Cd depletion in the rhizosphere of maize (B&C).

The results of model calculations showed an overestimation of the Cd influx. Therefore, a sensitivity analysis was conducted by incrementally changing the values of initial solution Cd concentration (C_{Li}) to find the reason for overestimation of Cd influx (Table 3-2).

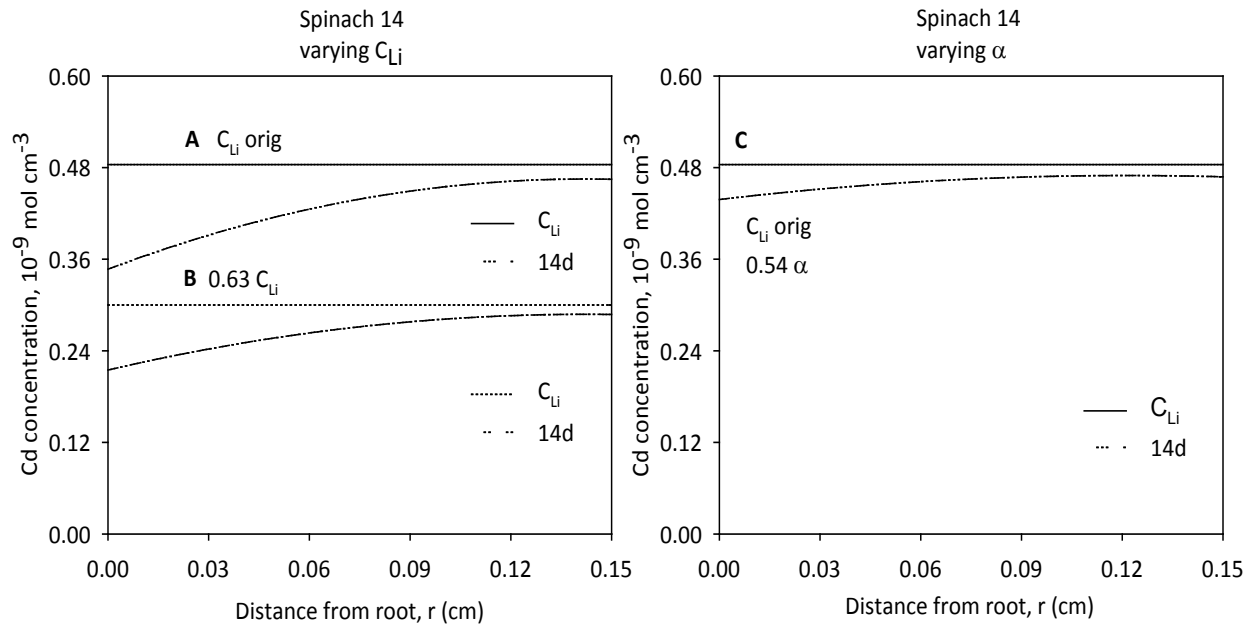


Figure 3-3 Calculated depletion of Cd from the rhizosphere of spinach after 14 days of uptake at 14 $\mu\text{mol Cd kg}^{-1}$ used NST 3.0 (A). Effect of changing C_{Li} or α value on calculated Cd depletion in the rhizosphere of spinach (B&C).

The sensitivity analysis for C_{Li} had shown that by decreasing the value down to 10% of the original value got 100% prediction of measured Cd influx of maize (Fig. 3-2B) and the C_{Li} decreased only by 0.012 μM . For spinach (Fig. 3-3B), the same was achieved by decreasing C_{Li} down to 63% of the original value and according to the model calculations, decreased solution Cd concentration at the root surface to 0.22 μM as compared to 0.019 μM for maize after 14 days of uptake.

On the other hand, when decreasing the root absorbing power (α value) of maize down to 10% of the original value caused an increased Cd concentration at the root surface from 0.22 μM to 0.24 μM (Fig. 3-2C). For spinach, calculated Cd influx was equal to measured Cd influx after decreasing the root absorbing power down to 54% but in contrast to maize the Cd concentration at the root surface decreased from 0.48 μM to 0.46 μM (Fig. 3-3C).

3.4 Discussion

Uptake-kinetic parameters from the nutrient solution experiment (Stritsis, 2005), together with soil Cd parameters (Tab. 3-1), were used in the NST 3.0 model. The model will help to understand why plants differ in their Cd accumulation because besides the calculated Cd uptake by plants, the Cd dynamics in the rhizosphere is calculated. Therefore, it will be possible, for example, to recognize to which extent different species lower the Cd concentration in the rhizosphere.

Before (Chap. 2) had shown differences in Cd uptake among the plant species mainly based on differences in their Cd influx. Changes of C_{Li} caused by the plants did not explain the lower Cd influx of maize and higher Cd influx of spinach. In this chapter, besides changes of C_{Li} caused by the roots we also included uptake properties of the root and used model calculation based on mechanisms of ion transport in soil and uptake kinetics of the root. Even though the model used is more comprehensive than considering just single factors, it was not able to describe the different Cd influx of the plant species, i.e. for one it overestimated the Cd influx up to 10 times and for another it was also not able to explain the differences of Cd influx observed among plant species.

These results are in contrast with results concerning major nutrients mainly K (Seiffert et al., 1995; El Dessouri, 2002) or P (Bhadoria 2002) where similar model calculation showed agreement between calculated and measured values or underestimated them even strongly. For other essential heavy metals such as Mn has been reported by Sadana et al. (2005) that the calculated Mn influx in different wheat cultivars and in low Mn soil concentration (below 1 μ M) reached only to 55% - 74% of the measured values.

Discrepancies between model calculated Cd influx could be due to (a) wrong estimation of soil or plant parameters or (b) not all processes occurring in the rhizosphere were included. Root absorbing power (α value) is a plant parameter that was calculated from study in solution culture (Stritsis, 2005). In nutrient solution experiment, Cd influx was linearly related to Cd concentration, and maize and sunflower had a similar influx but lower than flax and spinach (Stritsis, 2005 and Tab. 3-1) in the concentration range of interest, i.e., below 1 μM . Root absorbing power (α value) differed among plant species.

A further possibility is the initial soil solution concentration (C_{Li}) as soil parameter. It is a reliable model input parameter, which can be precisely measured. The displacement method permits accurate determination of the unaltered composition of soil solution (Adams 1974). Another difference to major nutrient is that there is almost no depletion of Cd in the rhizosphere, this as will be seen later is due to the much lower α value. In the lower concentration range, this relationship ($I_n = \alpha \times C_{L0}$) is approximately linear for both the Cd as well as for P, K and Mn but was much lower ($\alpha \approx 10^{-6} \text{ cm s}^{-1}$) than for P and K, which are in the order of $\alpha \approx 10^{-4} \text{ cm s}^{-1}$ (Sadana et al., 2005; Sayyari-Zahan et al., 2008). Therefore, sensitivity analysis for α , and C_{Li} should be conducted in order to find which parameter might have been wrongly estimated or influenced by plants in the rhizosphere. Another possibility to increase Cd soil solution concentration in the rhizosphere would have been through complexation of Cd in soil solution by plant root exudates.

Before we start the sensitivity analysis for α , C_{Li} should first run the model with the original data to calculate Cd uptake and Cd influx as well as the Cd depletion in the rhizosphere. In this way, the model provides a better understanding of factors

affecting the influence of Cd concentration on Cd uptake and causing the overestimation. Concentration profiles for Cd have not been calculated by other authors.

Figure 3-2A and 3-3A shows concentration profiles calculated using NST 3.0 at original C_{Li} and α value. The model predicted that after 14 days of Cd uptake caused a decrease of Cd concentration at the root surface of maize and spinach causing a concentration gradient that was necessary for calculated uptake. If plants had decreased Cd concentration at the root surface to a lower value, they would have obtained a higher Cd influx, because of a higher concentration gradient for diffusion. The reduction of C_{LO} was higher for spinach i.e. higher concentration gradient than that of maize but even so the model calculated for maize a 10-fold higher influx and for spinach of 1.5-fold higher, as measured. Obviously, this concentration ($0.19 \mu\text{M}$) at the root surface of maize was too high and explained the overestimation. The overestimation could be based on a high α ($1.2 \times 10^{-6} \text{ cm s}^{-1}$), too. For spinach, the reduction of C_{LO} to $0.35 \mu\text{M}$ was higher, but simulated closer the measured influx. The value of α ($3.2 \times 10^{-6} \text{ cm s}^{-1}$) is also high and 3-fold higher than that of maize but lead to better results. These results suggest that the higher Cd influx of spinach was due to its capability to decrease solution Cd concentration at the root surface to a relatively lower value, thereby increasing the concentration gradient and consequently, the diffusive transport of Cd to the root surface. For maize, concentration gradient was very small hence calculated uptake was lower in comparison to spinach. This shows that the Cd transport in soil actually would be able to satisfy an even much higher Cd influx but maize could not realize at this Cd concentration. Therefore, Cd transport in

soil is not limiting the uptake, but there is a close correlation between α value and the Cd influx.

The overestimate may be because the model does not consider the chemistry of Cd in soil solution such as chemical Cd mobilization (i.e. solubilization) or immobilization (i.e. insolubilization) and higher α value. Using sensitivity analysis, Sadana et al. (2005) found that underestimation based on lower soil-solution Mn concentration, C_{Li} , and the lower root absorbing power (I_{max} / K_m). Similar, in this experiment it is necessary to run a sensitivity analysis to find possible reasons for difference between calculated and measured Cd influx. The starting point for the sensitivity analysis was the dataset of Tab. 3-1. Of this, initial soil solution concentration (C_{Li}) and root absorbing power (α value) were gradually reduced (Fig. 3-1 and Tab. 3-1).

The sensitivity analysis shows that either, C_{Li} , or α value should be decreased to reach the measured influx. A reduction of C_{Li} at constant α should simulate a potential Cd complexation in soil solution caused by root activity. If Cd is taken up by plants as ion, C_{Li} reduction is designed to find out the soil solution Cd concentration (i.e. effective concentration), which may use the plant to obtain a calculated similar to the measured influx. By decreasing C_{Li} at constant α by a factor of 0.37 for spinach and 0.9 for maize, 100% agreement for Cd influx was achieved under $14 \mu\text{mol Cd kg}^{-1}$ addition (Fig. 3-2B and 3-3B). The model predicts that Cd uptake caused a decrease of Cd concentration at the root surface ($C_{Lo} = 0.019 \mu\text{M}$) of maize, but since the Cd influx was very small, that concentration gradient also was very small ($0.003 \mu\text{M}$) and was sufficient for calculated uptake. This shows that the soil is able to transport Cd to the root surface but maize cannot achieve a higher influx at this lower Cd concentration. For spinach,

concentration gradient was higher ($0.17 \mu\text{M}$) and obtained a higher influx. The effect of a decreasing C_{Li} on the Cd influx may be referred to less Cd depletion at the root surface leading to lower Cd influx. The decreasing C_{Li} by maize may have been a mechanism i.e. through complexation of Cd by root exudates in soil solution or at root surface.

From the above it is understood that the soil release Cd and therefore the different root absorbing power, i.e., α value, will influence the uptake of Cd from the soil. A reduction of α value should simulate the root physiology under low stress condition without reduction of biomass. Guide value of root absorbing power was taken from nutrient solution experiment (Stritsis, 2005). For maize, the root absorbing power was reduced 10 times, α value was found in the soil growing maize plants (Chap. 2; Fig. 2-4). At the root surface of maize, the soil solution Cd concentration was already very low ($C_{Li} = 0.22 \mu\text{M}$) with original α value (Fig. 3-2C), so by decreasing the α value to a lower value (90%), there was not a decrease at the root surface but, on the contrary, an increase of Cd concentration ($C_{LO} = 0.24 \mu\text{M}$). In this case, limiting factor for Cd uptake was not soil Cd transport but uptake kinetics, i.e. α value. This increase of C_{LO} at the root surface is due to a lower Cd influx than the transport caused by convective Cd transport in soil (mass flow).

In contrast, Cd concentration at the root surface of spinach ($C_{LO} = 0.48 \mu\text{M}$) was much higher, so that root was able to decrease the concentration ($C_{LO} = 0.46 \mu\text{M}$) further, thereby creating a small concentration gradient to allow diffusive transport of Cd towards the root and on the other side to decrease the calculated Cd influx.

Our previous results (Chap. 2) have shown that higher Cd influx was an important parameter for Cd accumulation in plants. In comparison to maize, spinach showed a high Cd shoot concentration even though the RA / SDW was low (Chap.2; Tab. 2-3) due to the high Cd influx. From the calculated concentration profiles around the root surface it is understood that the higher Cd influx in spinach was also due to its ability to reduce solution Cd concentration at root surface to a lower value as compared to maize. Nutrient uptake model NST 3.0 could not predict Cd influx and over-predicted Cd influx in all plants. The results showed that the Cd uptake was not explained without consideration of other not well-defined processes, such as chemical immobilization or complexation in soil solution (i.e. effective concentration). This high degree of sensitivity to C_{Li} and α for maize indicate that uptake kinetics (α value) was the major limiting factor for uptake and not Cd mobility through the soil towards the root. The amount of overestimation may indicate the dimension of the complexation influence, too. All plant species studied have different uptake kinetics and furthermore they seem to be able to change the composition of soil solution through root activity.

4 CADMIUM FRACTIONS IN SOIL AS AFFECTED BY PLANT GROWTH

4.1 Introduction

The results of Chapter 2 showed that plants vary in the amount of Cd they take up from soil. Main reason responsible for the variation in uptake was the Cd influx. The results furthermore showed that plants solubilized as well as immobilized Cd in soil. Model calculations (Chap.3) also indicated that Cd in soil solution may be partly present as chelate and not only as Cd²⁺ ion. This may indicate the ability of different plant species to influence and therefore, to access to different fractions of Cd in the soil.

The soil solution containing cations of elements such as Fe or Cd are in dynamic equilibrium with exchangeable cations sorbed to the solid phase or chelated to organic and inorganic ligands and it is most likely to be controlled by sorption–desorption reactions at the soil particle surface (Swift and McLaren, 1991). The results of Chapter 2 suggest that the change of Cd solution concentration caused by the root system resulting in maize and spinach to reduce and flax to increase it. This may be due to metal cations being released into soil solution from pools, which were not previously in rapid equilibrium with the soil solution. This change will affect the equilibrium of Cd in soil solution with Cd fractions in soil solid phase or its distribution, and its chemical form in the soil. On the other side, could due to the formation of Cd-compounds (organic or inorganic) in the rhizosphere, which has low solubility. This will be investigated by a fractionation of soil Cd.

Sorption of metal cations in soils is strongly influenced by soil characteristics and increased with increasing pH, redox potential, organic matter, cation exchange capacity, and contents of iron and manganese oxides (Lindsay, 1979; Herms und Brümmer, 1980; Kuo und Baker, 1980; Sposito et al., 1981; Backes et al., 1995; Serrano et al., 2005).

From measured Cd concentration in extracted fractions in which Cd is bound some empirical estimate of Cd availability for root uptake can be made, although little research has been examining plant uptake in relation to the chemical Cd fraction in soil. Many extraction schemes for heavy metals have been recommended depending on the soil characteristics, the soil contamination status, and may include up to five steps (Tessier, 1979) or even up to eight steps (Ma and Uren, 1998) in order to compare with other procedures. Other extraction design for heavy metals has been defined through a one-step soil-extracting procedure involving, for example, sodium nitrate, DTPA, or EDTA to determine the plant available amount (Lindsay and Norvell, 1978; Gupta and Aten, 1993; Ure et al., 1993).

The use of sequential extraction with a progressive increase in displacement strength provides additional information about soil Cd fractions that may be released from soil and become available for plant uptake because of root exudation and other soil processes, e.g., the Cd fraction that is mobilised if the pH is lowered to 4, or the fraction that may be mobilised from soil phase through root activity. Based on these partitioning into fractions, an assessment of plant availability and environmental influences of the heavy metals becomes possible, which cannot be derived from total element concentrations. Use of total concentration as a criterion to evaluate the potential effects on plant growth

suggest that all forms of a given metal have an equal influence on uptake; such an hypothesis is clearly unlikely.

This study focuses on possible changes in the chemical Cd fractions in soil due to the growth of maize, sunflower, flax and spinach. The aim was to establish whether the activity of plant roots changes the chemical binding of Cd in contaminated soils and whether changes of Cd binding in soil affect the concentration in soil solution. To characterize the Cd binding in soil a fractionation of soil Cd was conducted with the sequential extraction after Zeien and Brümmer (1989). Seven fractions are determined sequentially with increasingly strong extractants. These fractions are: mobile fraction (F.I), easily mobilizable fraction (F.II), occluded in Mn oxides bound fraction (F.III), organically bound fraction (F.IV), occluded in poorly crystalline Fe oxide bound fraction (F.V), occluded in crystalline Fe oxides bound fraction (F.VI) and residual fraction (F.VII).

4.2 Materials und Methods

Soil Cd fractionation was carried out on the soil samples from the pot experiment of Chapter 2 using the method of Zeien and Brümmer (1989). The soil Cd fractions are extracted in the following sequence: mobile fraction (F.I), easily mobilizable fraction (F.II), occluded in Mn oxides fraction (F.III), organically bound fraction (F.IV), occluded in poorly crystalline Fe oxide fraction (F.V), occluded in Fe oxides well crystalline fraction (F.VI) and residual fraction bound (in silicate) Cd fractions (F.VII) in to the bulk soil. The sequential extraction of the seven Cd fractions occurred on the same soil sample with a 7 step washing procedure.

Extraction procedure

I. Cd fraction: The mobile Cd fraction included water soluble and exchangeable (= nonspecifically adsorbed) Cd as well as soluble metallorganic Cd complexes. For this fraction 1.0 g dried, sieved (< 2 mm) of the soil sample was put in 50 mL acid-cleaned centrifuge beaker. 25 mL of NH_4NO_3 (1M not pH adjusted) was added and the beaker was shaken overhead for 24 hours. After centrifugation for 10 minutes at 2500 (r/min) at 20°C the solution was decanted over folded filter in 100 mL polyethylene bottles and the filtrate stored at 6°C. For stabilization of the filtrates 0.25 mL conc. HNO_3 were added.

II. Cd fraction: In centrifuge beaker to the remaining soil sample 25 mL NH_4OAc (1M adjusted to pH 6 with acetic acid) was added to extract the easily mobilizable Cd fraction. The II fraction contains Cd specifically adsorbed, occluded close to particle surfaces, bound to carbonates and extracted from metal-organic complexes of low stability. The solution with NH_4OAc was shaken overhead for 24 hours and centrifuged for 10 minutes at 2500 (r/min) at 20°C. The supernatant was decanted over folded filter in 100 mL polyethylene bottles and the remaining rest solution was twice washed (10 min overhead shaken) with 12.5 mL of NH_4NO_3 (1M not pH adjusted). The supernatant was each time decanted again in the 100 mL polyethylene bottles (filtrates were united). To the filtrates 0.25 mL conc. HNO_3 were added to stabilize the solution.

III. Cd fraction: After extraction of fraction II, 25mL solution consisting of $\text{NH}_2\text{OH-HCl}$ (0.1M) and NH_4OAc (1M) adjusted to pH 6 with 5M HCl were added to the centrifuge tube to extract the III Cd fraction. This fraction is considered occluded in Mn oxides plus residual specifically adsorbed and small proportions of

organically bound heavy metals. The beaker was shaken overhead for 30 min and centrifuged for 10 minutes at 2500 (r/min) at 20°C. The supernatant was decanted over folded filter in 100 mL polyethylene bottles and the remaining soil was two times washed (10 min overhead shaken) with 12.5 mL (1M NH_4OAc adjusted to pH 6 with acetic acid). The supernatant was each time decanted again into the 100 mL polyethylene bottles (filtrates were united). Into the filtrates 0.25 mL conc. HNO_3 were added to stabilize the solution.

IV. Cd fraction: The extractant for this fraction consisted of $\text{NH}_4\text{-EDTA}$ (0.025M) and NH_4OAc (1M) adjusted to pH 4.6 with acetic acid to get the organically bound Cd. 25 mL of this solution were added into centrifuge beaker, overhead shaken for 90 min and centrifuged for 10 minutes at 2500 (r/min) at 20°C. The supernatant was decanted over folded filter into 100 mL polyethylene bottles. To recover the solution with the heavy metals remaining in the soil sample, the soil sample was mixed with 12.5 mL of 1 M NH_4OAc (adjusted to pH 4.6 with acetic acid), overhead shaken for 10 min, then centrifuged and filtered. The collected filtrates were united and stored at 6°C.

V. Cd fraction: To extract the Cd assumed to be occluded in poorly crystalline Fe-oxides, the soil sample remaining in the centrifuge beaker was mixed with 25 mL of 0.2 M $\text{NH}_4\text{-oxalate}$ solution (0.2 M di-ammonium oxalate monohydrate and 0.2 M oxalic acid dihydrate with ammonia solution adjusted at pH 3.25) overhead shaken for 4 hours in darkness and centrifuged for 10 minutes at 2500 (r/min) at 20 °C and the supernatant was decanted over folded filter in 100 mL polyethylene bottles. In a subsequent wash, 12.5 mL of 0.2 M NH_4 oxalate solution were added in the remaining soil sample, overhead shaken for 10 minutes in darkness then

centrifuged and filtered as above. Then the resulting filtrates were united and stored at 6°C in darkness.

VI. Cd fraction: To the soil sample remaining in the centrifuge tube 25 mL 0.1 M of ascorbic acid and 0.2 M NH_4 oxalate solution (adjusted at pH 3.25 such as Cd fraction V) were added for extraction of Cd occluded in crystalline Fe-oxides. After adding of extracting agent, sample was treated for 30 minutes in water bath at $96 \pm 3^\circ\text{C}$. Following was centrifuged for 15 minutes at 2500 (r/min) at 20°C and the supernatant was decanted over folded filter in 100 mL polyethylene bottles. For washing, the soil sample was shaken overhead once with 12.5 mL of 0.2 M NH_4 oxalate solution (adjusted at pH 3.25 such as Cd fraction V) for 10 minutes in darkness, then centrifuged and filtered as above. Then the filtrates were united and stored at 6°C in darkness.

VII. Residual fraction: As the last fractionation step, the soil sample is subjected to total digestion. The soil sample remaining in the centrifuge beaker is transferred to a 250 mL glass flask and then 10.5 mL of concentrated HNO_3 (65%) and 3.5 mL of concentrated HCl (37%) were added. To avoid uncontrolled reaction processes during the digestion process, samples were pre-treated 45 min at 70°C , than 30 min at 110°C and finally 180 min at 120°C until it reached a whitish-gray appearance of the soil sample. The residue was transferred with water into glass flasks and filtered in 100 mL polyethylene bottles. All filtrates of extracted solution were analyzed for Cd concentration using graphite furnace atomic absorption spectrometer GFAAS. The limits of detection were $2 \mu\text{g L}^{-1}$. Total Cd content in soils was determined by digesting 0.2 g of dried soil with 3 mL of HNO_3 (65%) in teflon box under high pressure at 180°C followed by analysis.

The Cd determinations were always performed with D2 deuterium background correction and with graphite tube and platform. During the Cd determination with GFAAS significant matrix problems occurred which were primarily attributable to the use of highly concentrated salt solutions. The problem of the high concentrated salt solutions could often be improved through strong dilution. The optimization of measurement parameters (temperature control and atomization time) brought a reduction of matrix problems or improved the reproducibility (Tab.8-2).

For more details of the extraction procedure and characterization of the different Cd fraction see Zeien and Brümmer (1989) and Zeien (1995).

4.3 Results

Figure 4-1 shows that plants growth affected the Cd content of the different Cd fractions, causing not only Cd depletion but also an increase, which could be described as a Cd immobilization. These effects varied among plant species. Fraction I (the mobile Cd) was decreased by all species, this decrease was in tendency smaller for maize than for the other species.

In F.II (easily mobilizable Cd) maize and spinach increased while flax decreased the content of this fraction and sunflower had no effect. Fraction III (Cd occluded in Mn oxide) was not affected by maize and flax while sunflower and spinach reduced its content. The organically bound Cd (F.IV) was increased by maize and flax but sunflower and spinach had no affect on it. The amount of Cd present in V, VI, VII fractions was only around 5% of total Cd and was therefore not considered separately (Appendix Tab.8-1).

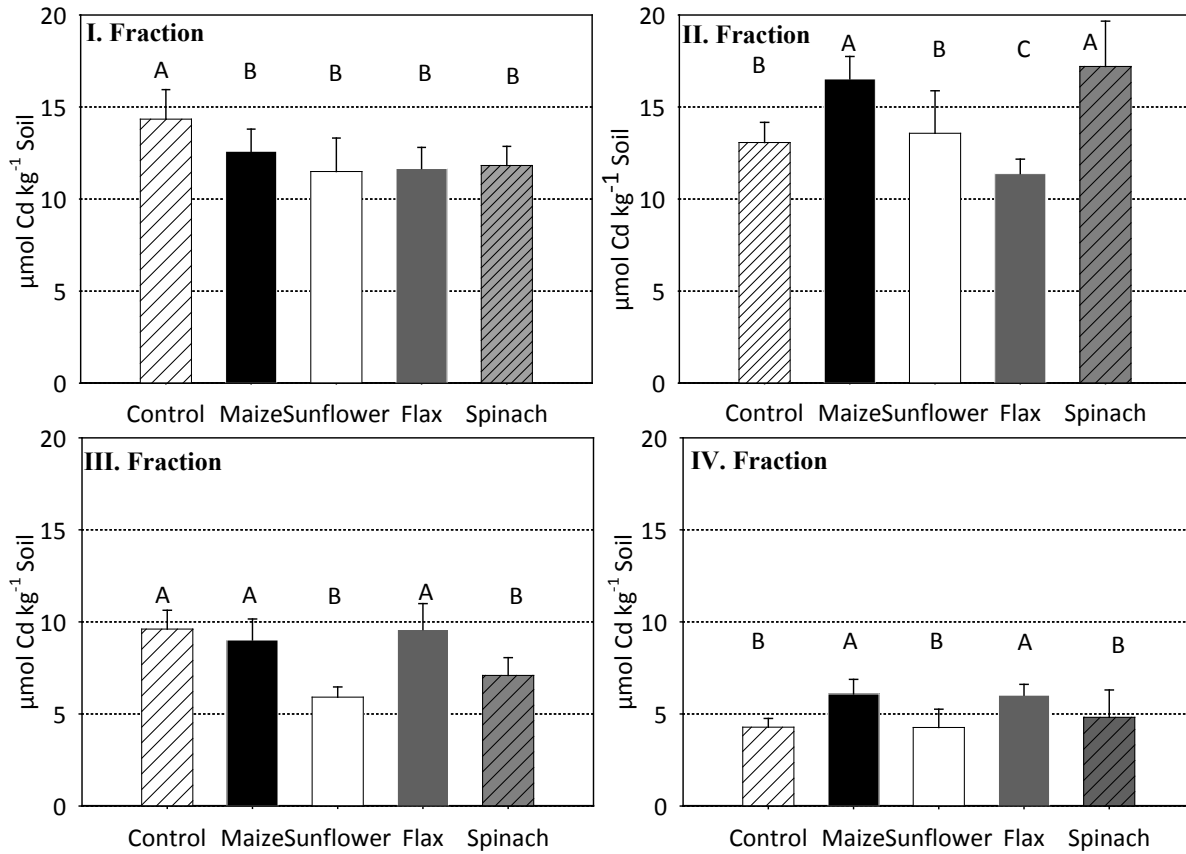


Figure 4-1 Cadmium in the first four fractions by comparison at 40 $\mu\text{mol Cd kg}^{-1}$ addition in soil. Different upper case letters show significant differences among plant and fractions ($p < 0.01$; Student-Newmann-Keuls method).

Figure 4-2 shows the distribution of Cd among the different fractions as affected by plant growth. In control soil, Cd was mainly found in the mobile and easily mobilizable fraction (64%). Significant concentrations of Cd were found in F.III (22%) and F.IV (10%). Fractions V, VI, VII amounted to less than 5% of their total Cd concentration (Appendix Tab. 8-2). The plants have a different profile in comparison to control.

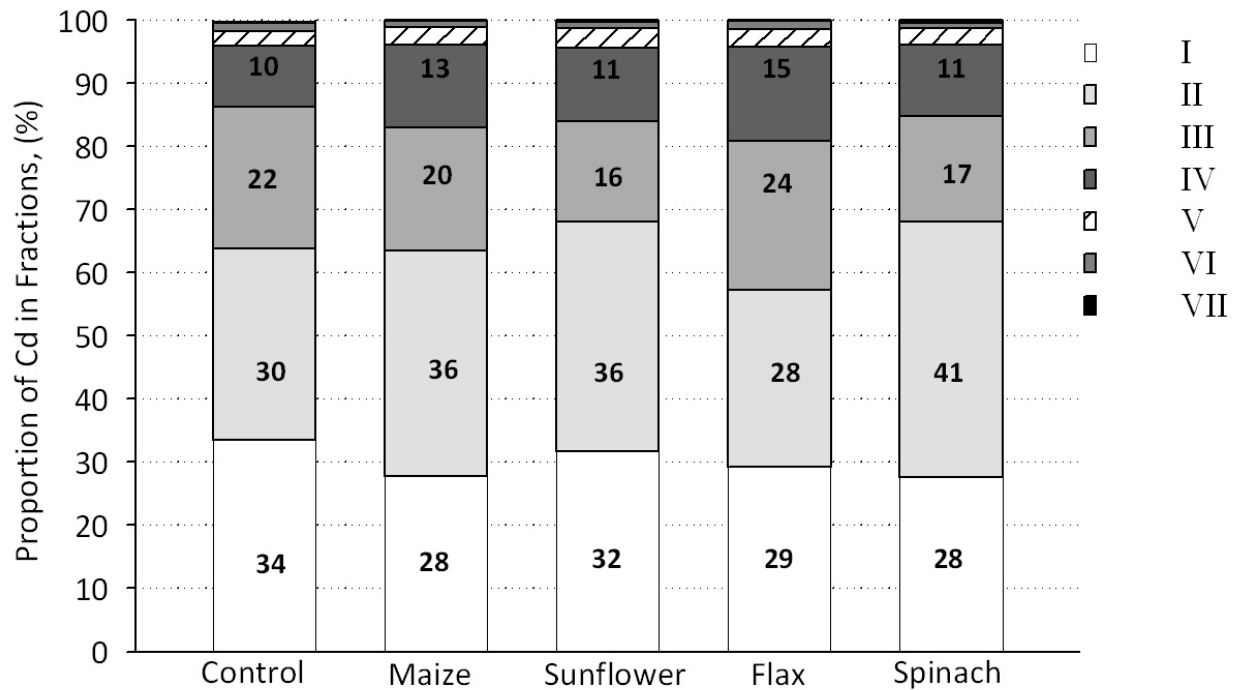


Figure 4-2 Percentage distribution of Cd in the run of the experiment on the 7 fractions of sequential extraction in soil after $40 \mu\text{mol kg}^{-1}$ Cd addition.

For spinach and sunflower about 70% of Cd was found in F.I and F.II while flax had only 57%. In maize and sunflower, the F.II increased slightly (about 6%) whereas in spinach it increased more (11%). Flax had similar Cd content as the control in the F.II but a decrease of 6% in the F.I. Concomitant, the contents of Cd in the F.III were a little changed by maize and flax. Remarkable is the increase of the F.IV in maize (3%) and flax (5%) in comparison to the control. Fractions V, VI, and VII together contain less than 5% of the total Cd.

In Fig. 4-3, the Cd uptake expressed in $\mu\text{mol Cd kg}^{-1}$ soil is compared with the changes of Cd content of the different Cd fractions, also expressed in $\mu\text{mol Cd kg}^{-1}$ soil. The lowest Cd uptake was found in maize and rose significantly from sunflower and flax to spinach. Looking at the change of the Cd content in each fraction, then the mobile fraction shows the biggest changes. Here, the decrease

in Cd content of fraction I except maize ($1.6 \mu\text{mol Cd kg}^{-1}$) were comparable higher (about $2.5 \mu\text{mol Cd kg}^{-1}$).

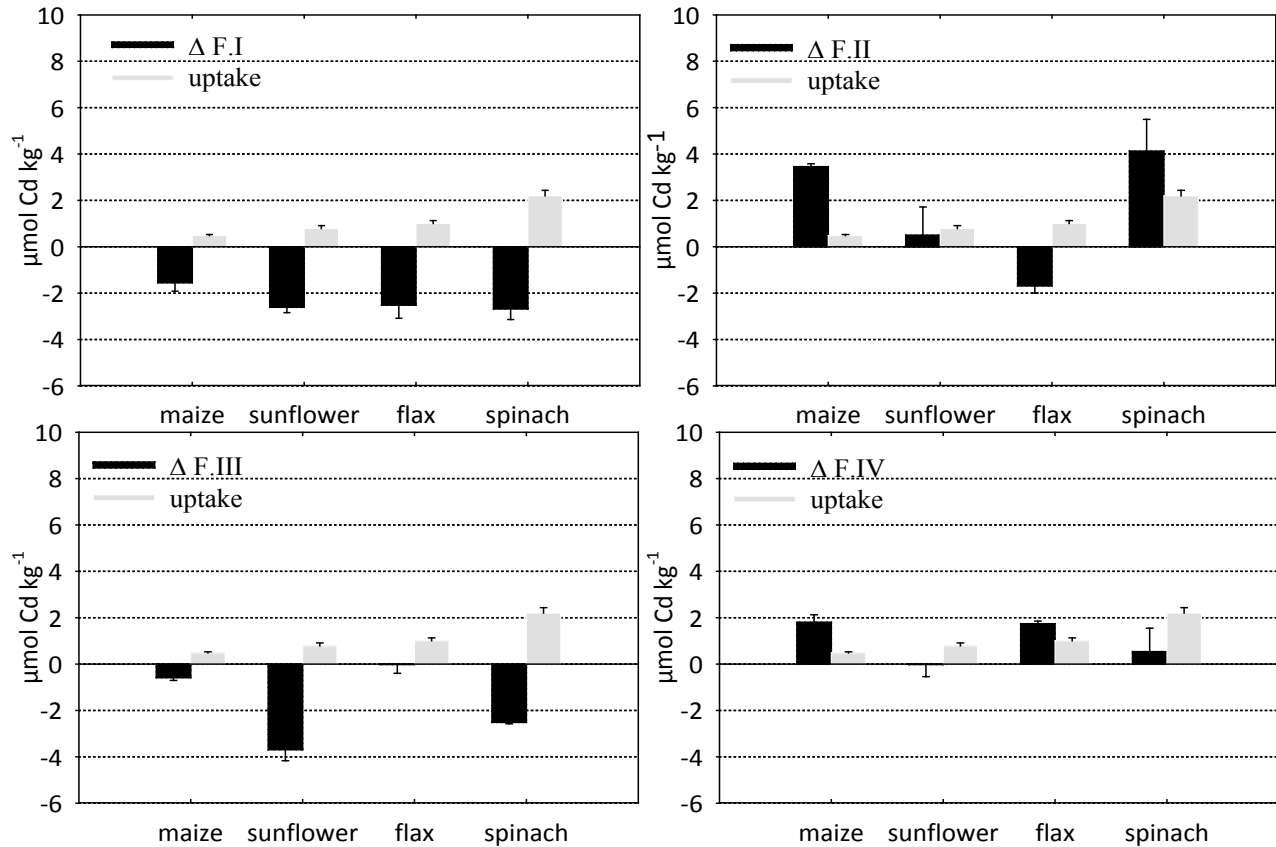


Figure 4-3 Comparison of Cd uptake by different plants species with the change (Δ) of Cd in soil Cd fractions I, II, III, IV caused by plant growth.

The plant effect on the Cd content in fraction II was very different, i.e. it increased and only with flax ($1.7 \mu\text{mol Cd kg}^{-1}$) it decreased. For fraction III, the Cd content decreased strongly in sunflower ($3.7 \mu\text{mol Cd kg}^{-1}$) and spinach ($2.5 \mu\text{mol Cd kg}^{-1}$), while in maize and flax there was no change. In fraction IV the Cd content increased clearly in maize and flax ($1.7 \mu\text{mol Cd kg}^{-1}$) while in spinach the increase was very low and sunflower had no effect.

Cadmium in soil solution (Chap. 2; Fig. 2-3) as well as the different Cd fraction varied strongly among plant species. Since Cd in soil solution is in equilibrium with Cd in the solid phase we tried to find out which of the fraction might be controlling Cd concentration in the soil solution.

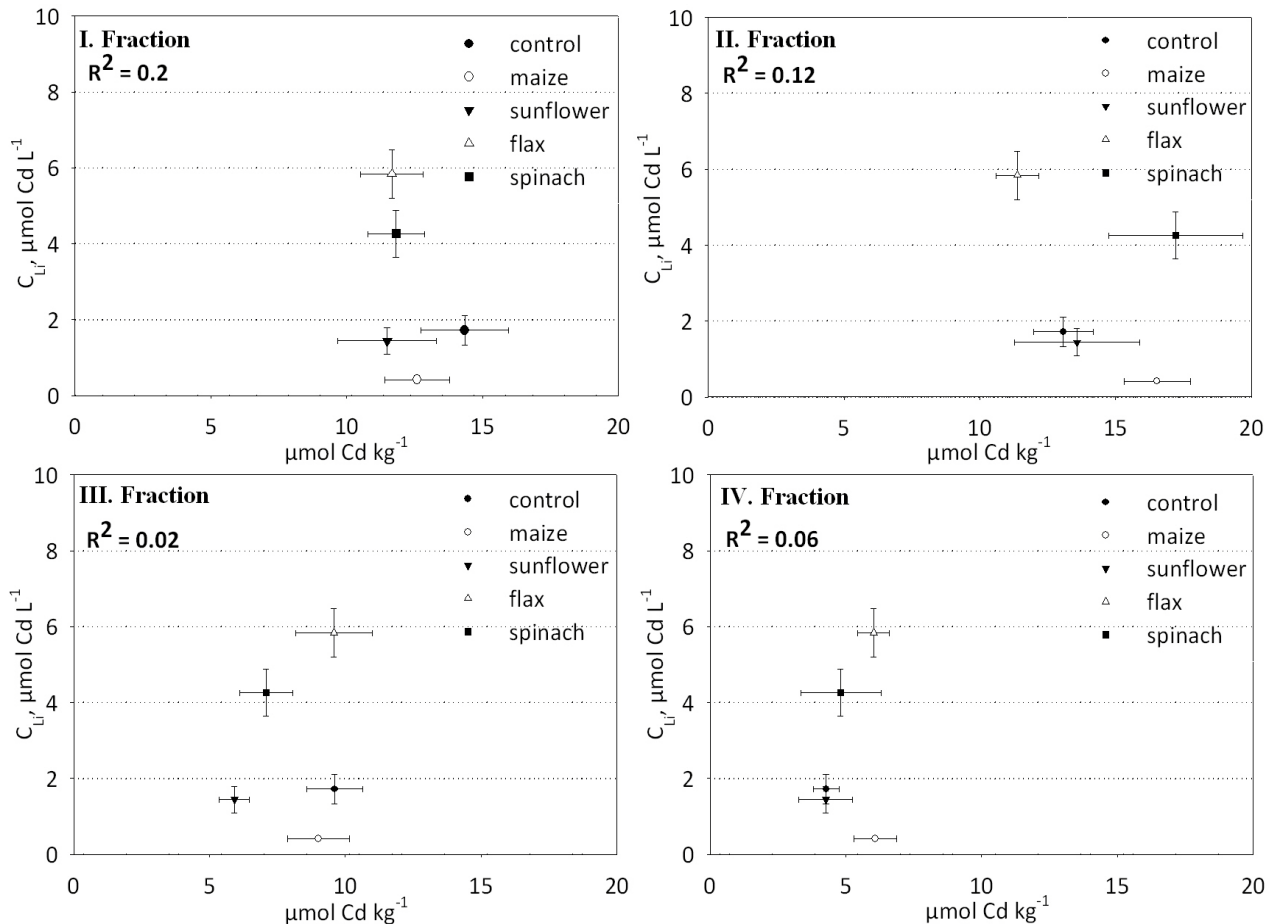


Figure 4-4 Relationship between Cd concentration in soil solution, C_{Li} , at second harvest and Cd contents in Cd fractions I, II, III, IV.

Figure 4-4 shows the relationships between the Cd in soil solution and the Cd content in the different fractions. With r^2 -values from 0.02 to 0.2 shows that there is no clear relationship between the soil solution concentration and the total content of any of the fractions. Spinach and flax have always more Cd in the soil solution as maize and sunflowers. Since according to model calculation (Chapter 3)

the ionic Cd concentration in soil solution was much different from total Cd in soil solution we also plotted the Cd concentration as estimated by the model against Cd content of the different Cd fractions. This is shown in Figure 4-5. It can be seen, the correlation coefficients for Cd obtained are better than with the original concentration but as in the C_{Li} (Fig. 4-4) no relationship could be established for Cd with either soil Cd fraction. It should also be noted that ammonium nitrate extraction resulted in much better correlations ($r^2 = 0.45$) for Cd than in the other fractions.

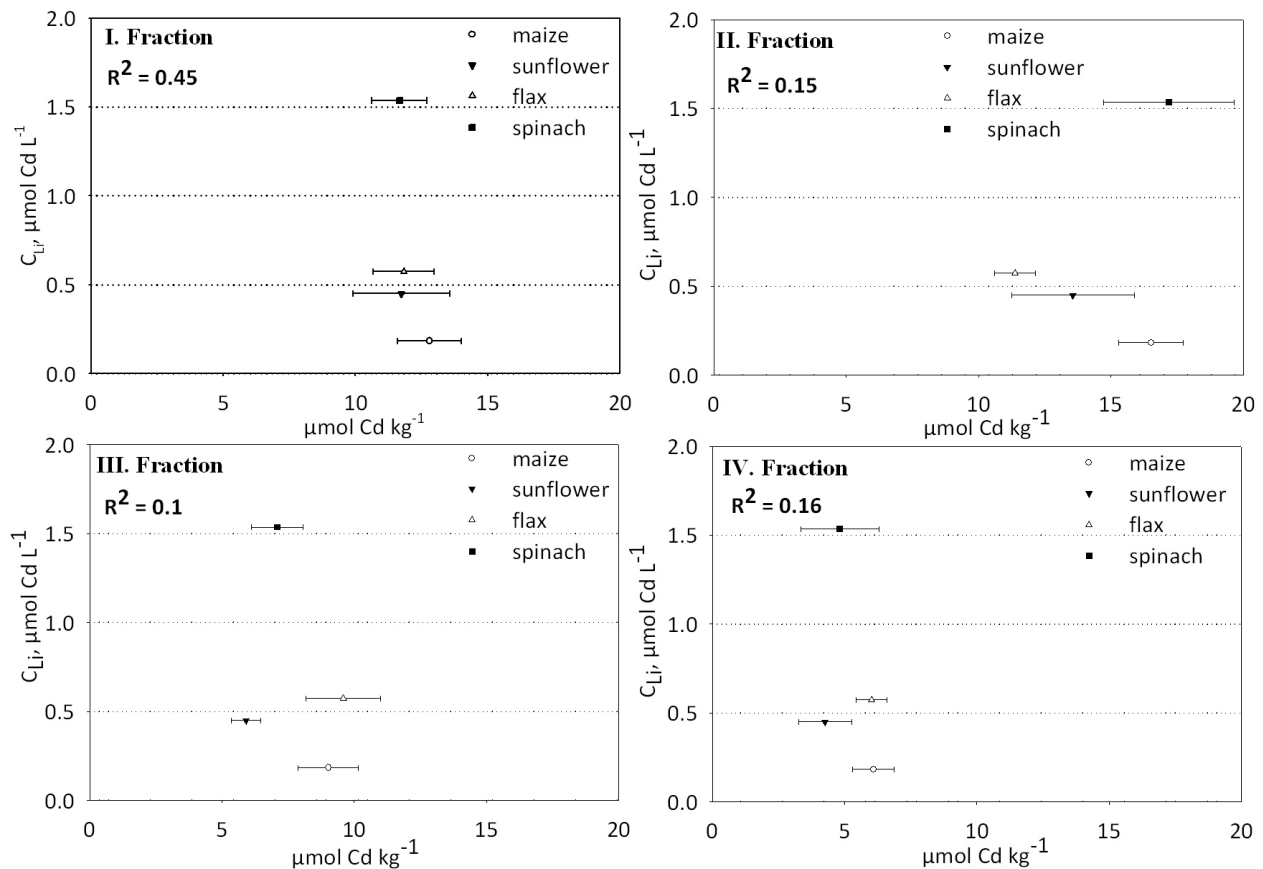


Figure 4-5 Relationship between the Cd content in Cd fractions I, II, III, IV and the varying concentration, C_{Li} , of Cd in the soil solution (average between first and second harvest) getting from sensitivity analysis. C_{Li} is the Cd concentration in solution at which calculated and measured Cd influx was equal.

4.4 Discussion

Cadmium distribution among specific chemical forms in soil varies widely because soils consist of heterogeneous mixtures of organic and inorganic solid components as well as a variety of soluble substances and based on the Cd chemical properties and soil characteristics. With the sequential extraction, it is intended to understand the interactions between Cd and soil components, as well as to determine the distribution of Cd among binding forms. The relative binding strength of Cd at different solid phases can estimate the potential reactivity of Cd under different environmental conditions and thus identify the environmental effectiveness of Cd in soil. The sequential extraction used in this study was developed to assess the form of Cd binding in the soils (Zeien, 1995) and the mobility (i.e. extractability) is defined as the extracted Cd amount from organic and inorganic solid components. Hence, sequential extraction can give an indication of the binding strength of Cd in soil and its potential for distribution over time due to changes in soil chemistry. The seven binding fractions of Cd are operationally defined by an extraction sequence that follows the order of increasing acidity with extractants of different complexing and redox properties (Zeien and Brümmer, 1989; Zeien and Brümmer, 1991; Zeien, 1995). The advantage of this method over other, is that it was developed to determine heavy metal binding form in soils with oxidizing conditions and with less than 5% carbonate content. A further advantage of this method was the minimization of matrix problems as compared to the previously proposed methods for sequential extraction procedure (Zeien, 1995). It should also be noted that this sequential extraction procedure presents certain analytical problems that lead to deviation from the actual amount of Cd added to soil. However, comparison of the sum of the Cd concentrations in the individual fractions with the total Cd concentration

shows satisfactory agreement for plants. From the Tab 8-1 in the Appendix, there is a deviation (5-15%) of the sum of fractions of the sequential procedure in relation to the total amount of Cd that was determined separately with acid digestion (65% HNO₃). Similar problems and deviation associated with the selectivity of the reagents and the occurrence of analytical problems during this sequential extraction have been observed and commented on other investigations (Zeein 1995; Keppler 1999; Keller 2000; Domeyer 2006).

Cadmium concentrations of individual fractions of the sequential extraction analysis are shown in Fig. 4-1 both with plant growth and without. The amounts of Cd extracted by the seven extractants were different without plants. Supposing that bioavailability is related to binding forms, then Cd bioavailability increases in the order: Fraction VII (F.VII) < Fraction VI (F.VI) < Fraction V (F.V) < Fraction IV (F.IV) < Fraction III (F.III) < Fraction II (F.II) < Fraction I (F.I). This order is just a generalization and shows only qualitative information about Cd bioavailability and mobility (i.e. extractability). The mobility in soils is determined by solution and precipitation reactions as well as adsorption and desorption reactions in conjunction with complexation processes of organic and inorganic substances. Cadmium is bound in soils, both specific and nonspecific to the surfaces of many adsorbents with variable adsorption capacity and binding strength. In the sandy soil enriched with 40 μmol Cd kg⁻¹, it was mostly concentrated in F.I and F.II, although it was also present in other fractions (Fig. 4-1). The high proportion of Cd in F.I (14.3 μmol Cd kg⁻¹) and in F.II (13.1 μmol Cd kg⁻¹) in soil without plants reflects the greater tendency of Cd to bind as exchangeable in this soil. The Cd in F.I is primarily non-specifically adsorbed by electrostatic attraction of negatively charged surfaces (Brümmer et al., 1986; Sposito 1998, pp127-147;

Scheffer/Schachtschabel 2002, pp378-382). The use of electrolytes, such as NH_4NO_3 , in the extraction solution achieves displacement of Cd ions held by electrostatic attraction to negative sites on particle surfaces. The 1M NH_4NO_3 fraction is in a concentration-dependent relationship on the one hand to the soil solution and the other hand to the stronger binding forces specifically bound Cd fractions. These provide with modified pH either a replenishment pool or a provisional fractional accumulation of Cd. In sandy soil occurred increased F.I because of the strongly acidic soil reaction (pH 4.4 - 4.5) and absorbed area of organic matter (C_{org} 2.8%) that they have a high pH-dependent binding capacity for Cd.

The extraction capacity of 1M NH_4OAc solution (pH 6.0) of Cd F.II to extract specifically adsorbed Cd is based on the dispersing ability of the NH_4^+ cation and the complexing ability of the acetate anion. The NH_4OAc solution displaces Cd close to surface binding sites of clay minerals and oxides, carbonates bond forms, and organometallic complexes of low binding strength (Herms and Brümmer, 1984; Brümmer et al., 1986; Zeein 1995). With increasing soil acidification a shift of the binding affinities from higher towards lower binding strength, might be occurred i.e. unspecific adsorption leading in an increased proportion of mobile Cd (Zeein 1995). The pH significantly influenced the sorption behavior of soil substances so that can also contribute slightly soluble organometallic complexes in a medium acidic pH range (5.0 - 5.9) to an increased Cd solubility (Herms und Brümmer, 1978; Zeien 1995). Furthermore, Cd desorption in soil increases significantly at pH < 6.5 (Herms and Brümmer 1980; Ratuszny et al., 2009). From the above it is understood that on the adsorbent surface is more likely to present

a continuum rather than a sharply defined change between binding sites of different binding energies (Tiller et al., 1984a, 1984b).

In this study, the Cd bound in F.III in control soil was $9.6 \mu\text{mol Cd kg}^{-1}$ (22%). Manganese in soils under aerobic conditions is mainly present as Mn-oxides and hydroxides as well as in Mn-Fe mixed oxides with high surface activity, which can affect the Cd content in soils because are able to bind strong Cd (Brümmer et al., 1983; Sposito 1998; Scheffer/Schachtschabel 2010, p136). The use of 0.1 M $\text{NH}_2\text{OH-HCl}$ + 1 M NH_4OAc at a pH of 6.0 is based on the reductive dissolution of Mn oxides under consideration to a good separation of Cd bound in Fe-oxides (Chao 1972; Zeein 1995).

The Cd bound to organic matter in F.IV was low ($5 \mu\text{mol kg}^{-1}$ or 10%). Cadmium associated with organic substance is either adsorbed or complexed. By using the strong complexing agent 0.025 M $\text{NH}_4\text{-EDTA}$ combined with 1 M NH_4OAc at pH 4.6, a selective detection of Cd bound to stable organic complexes is obtained. The superiority of 0.025 M $\text{NH}_4\text{-EDTA}$ is due to a complete complexation of organic-bound Cd fractions (Zeein 1995).

According to Ratuszny et al. (2009) using the same extraction procedure, Cd was mainly found in F.I (27.6%), F.II (26.9%) and F.III (25.0%) in acid soils (pH 5.0-6.5). The same extraction order, $\text{F.I} > \text{F.II} > \text{F.III}$ (about 75%), was also reported for other forest soil with pH 5.04 (Keppler, 1999).

Sequential extractions might give the information needed to explain different uptake efficiencies for different metals such as Cd. Figure 4-2 shows the relative change of the respective Cd fractions by plants while Figure 4-3 points the relationship between Cd uptake and change of Cd soil fractions of plants in

comparison to control soil. For all plants, the first four fractions were changed by 5% on average. Comparisons in Fig. 4-1 and Fig. 4-3 indicate that after 43 days of germination, plants reduced Cd associated with F.I in the soil. The negative change was remarkable because F.I could have positive influence on Cd release into soil solution and therefore could explain the Cd uptake of all plants. However, we are not sure whether the entire Cd was correlated with Cd uptake, because there is no clear relationship between Cd content in F.I and Cd concentration in soil solution (Fig. 4-4 and see below). To this, Brümmer et al. (1998) emphasize that F.I on lower contaminated soil was less meaningful for transfer into the plant. Further studies by Singh and Myhr (1997) causes an increased Cd addition to the soil high levels of NH_4NO_3 -extractable Cd, but this relationship was not confirmed in the Cd contents in plants.

On the other hand, the Cd content of soil planted with maize and spinach increased the content of F.II indicating a change in the Cd distribution (Fig. 4-1). In Figure 4-3 is only recorded a negative change of Cd in the soil grown to flax ($1.7 \mu\text{mol kg}^{-1}$) whereas in maize ($3.5 \mu\text{mol kg}^{-1}$) and spinach ($4 \mu\text{mol kg}^{-1}$) is noticed a positive change. The F.II of soil planted with maize and spinach was observed a high bond of Cd that could be explained by the high extractability of Cd using the 1M NH_4OAc (pH 6.0). This extraction behavior of 1M NH_4OAc (at pH 6.0) is characterized by a partial extraction of organometallic complexes of low bond strength (Zeein 1995) which may be derived from the root. Results of Gransee et al. (1998) show that root exudates of maize were composed to 75% of sugars, to 17% of amino acids and amides and to 8% of carboxylic acids organic substance with low adsorptive capacity. Keller (2000) reported that the dominant component in the acid samples of spinach (Monnopa) was oxalic acid. In addition,

it could find with comparable mean excretion rates of citric acid, malic acid, malonic acid, succinic acid and lactic acid. In addition, small amounts were at 2-oxoglutaric, acetic acid and fumaric acid. The difference organic acid profile could be an explanation for the higher amount of Cd extracted from F.II of maize and spinach samples and lower of flax samples indicating a root exudation that caused a different Cd content in comparison to control soil. The negative change in F.II ($1.7 \mu\text{mol kg}^{-1}$) of soil planted with flax is similar to the positive change in F.IV ($1.7 \mu\text{mol kg}^{-1}$) whenever there is a distribution of Cd. The distribution could be explained with a different exudation profile i.e. exudation of substance with high molecular weight. Besides, in case of flax the change in F.II could explain the Cd uptake while for maize and spinach it is not possible.

Effect on the Cd quantity was found in extraction of F.III, too. The reductive dissolution of Mn oxides caused from $0.1 \text{ M NH}_2\text{OH-HCl} + 1 \text{ M NH}_4\text{OAc}$ at a pH of 6.0 can result in complete or partial dissolution of any Mn oxides present, a dissolution process that can release Cd ions associated with these soil components. Among plants, the Mn oxide fraction contained the greatest amount of Cd in control soil, maize and flax in comparison to sunflower and spinach (Fig. 4-2). It is remarkable that Cd in soil of sunflower and spinach showed a low value in the F.III of the procedure in comparison to control soil. A low proportion of Cd in the fraction bound to Mn oxides indicates that probably a considerable change in the oxygen content (e.g. due to root or bacterial activity during the vegetation period) can reduce the redox potential in rhizosphere to values sufficiently low to achieve reduction of Mn and therefore to affect the Cd content. In the rhizosphere of sunflower, field beans and peas Fischer et al. (1989) as well as Flessa and Fisher (1992) found root-induced reduction reactions while in the

rhizosphere of wheat and oat showed no change in redox potential. Observing the changes of Cd will confirm a significant reduction in sunflower ($3.7 \mu\text{mol kg}^{-1}$) and a little in spinach ($2.5 \mu\text{mol kg}^{-1}$).

A negative change of Cd content indicates that the rhizosphere of sunflower and spinach could affect the Cd content. For Cd uptake, we can not certainly draw conclusions because both the F.III and the F.I could cover Cd uptake of sunflower ($0.8 \mu\text{mol kg}^{-1}$) and spinach ($2.2 \mu\text{mol kg}^{-1}$). It is also difficult to find a distribution of Cd especially in the case of sunflower. The negative change in the maize ($0.6 \mu\text{mol kg}^{-1}$) was almost similar to the Cd uptake (Fig. 4-3). This change was enough to explain the Cd uptake for each plant.

The results from Fig. 4-3 show that plants influenced F.IV but not in the same way as the F.III. Use of $0.025 \text{ M NH}_4\text{-EDTA}$ and $1 \text{ M NH}_4\text{OAc}$ (pH 4.6) released substantially more Cd from both soils grown to maize and flax than from soil without plants (Fig. 4-1). This extractant is able to release organically bound Cd by solubilizing a proportion of the organic matter. In maize and flax soil, 5% of the total Cd was released by $0.025 \text{ M NH}_4\text{-EDTA}$ and $1 \text{ M NH}_4\text{OAc}$ (pH 4.6), reflecting the fact that an amount of organic matter could have been added to the soil in the form of organic plant exudates. The composition and amount of root exudates depends on several factors such as plant species, plant age and stage of development, plant growth and presence of microorganisms in the rhizosphere soil. In soils, the organic matter consists of complex polymeric material known as fulvic and humic acids (with high adsorptive capacity) and non-humic substances such as mucilage (polysaccharides), proteins, sugars, organic acids, peptides, amino acids and fats (Egle 2002; Bais et al., 2006; Scheffer/Schachtschabel, 2010 p56-58). These natural complexing agents include root exudates, which can

reduce mobility (i.e. extractability) due to interaction with Cd ions. There are several indications that considerable proportions of Cd are still bound at pH values between 3 and 4 (Herms and Brümmer, 1984; Brümmer et al., 1986). Unlike spinach and sunflower, F.IV for maize and flax has an important role when assessing the Cd extraction. With decreasing pH increases the extraction of Cd at pH 4.6 only slightly in maize and flax. In this fraction the change was for both plants positive and similar ($1.7 \mu\text{mol Cd kg}^{-1}$). Obviously, the Cd uptake could not be affected due to high Cd content bound on F.IV.

To study the connection of the Cd concentrations present in the soil solution with the different Cd fractions, the results of sequential extraction were compared with Cd soil solution concentration obtained from modified displacement method according to Adams (1974). In contrast to Cd reactivity at different solid phase, relatively few publications deal with interaction between Cd concentration in solid phase and Cd concentration in solution after growing of plants. Because the plant properties affect the composition of soil solution it is important to evaluate the bioavailability and mobility of Cd to understand chemical behavior and change of Cd contaminants in soils.

It should be noted that the classification of this extraction scheme as geochemical “mobilisable” and geochemical “easily mobilizable” Cd is valid only under certain laboratory conditions (pH, extractants), because Cd bound in soils under natural environmental conditions can potentially be released into solution from other fractions. The very low Cd concentration in the soil solution (varied between 0.4-5.8 μM) represents a further difficulty to achieve a meaningful relationship between mobile Cd fraction and Cd in the soil solution (Fig. 4-4 and Tab. 8-3).

Cadmium is non-specifically adsorbed in the low pH range (3-6) and is thus easier to bring into solution (Zeien 1995; Keller 2000). Brümmer et al. (1986) suggested that, especially in acid soil, the composition of the soil solution might be determined mainly by adsorption-desorption processes of Cd combined with complexation processes of organic and inorganic ligands. Also only insufficient information exists about the availability of organic Cd complexes in the solution phase. This lead to different assessments of Cd relationships related to the enrichment in the soil solution (Gupta and Sinha, 2006).

The fact that the Cd concentration in soil solution (Chap.2, Fig. 2-3) decreased (Maize, Sunflower), but on the other increased (spinach, flax) could indicate a collection of different Cd fractions in soil. Unfortunately, anything comparable can be found in the literature, so that this issue need be explored with further research. Under the assumption that Cd is present in soil solution complexed or chelated, then the effective Cd soil solution concentration must be much less. The results from the sensitivity analysis (Chap.3) provide very low value of Cd concentration, so that clear relationships make even more difficult (Fig. 4-5). In the present experiment, the Cd concentrations in soil solution after addition of 40 $\mu\text{mol Cd kg}^{-1}$ varied between 0.74 μM and 4.6 μM . If we consider the result of sensitivity analysis then 50% to 90% of Cd in soil solution may be complexed or chelated (Chap. 3, Fig. 3-1, and Tab. 3-3) and the effective concentration of Cd^{2+} ions in solution will be even lower than 0.15 to 1.6 $\mu\text{mol L}^{-1}$. Sayyari-Zahan et al. (2010) reported that the Mn concentration in soil solution should be only 3% to 20% of Mn in solution. This method recommend for the sequential extraction of Cd are based on the assumption that the above-mentioned binding forms exist and depending on pH enrich a part of Cd in the soil solution. Release of Cd into the

soil solution depends on strong depletion of the soil solution, composition of soil solution, decomposition, oxidation of organic matter and root activity in rhizosphere. The mechanisms by which plants are able to increase (spinach, flax) or decreased (Maize, Sunflower) Cd in to soil solution are high or low root activity as well as high or low influx.

Concluding, the results show that there is no clear relationship between the soil solution Cd concentration and Cd levels in any of the Cd fractions (Fig. 4-3). However, not the entire Cd in soil is available for plant use. Due to the weak binding of Cd in the soil and the largest amount of Cd found in the F.I can be concluded that those enriched the soil solution in higher percentage. However, in multi component-systems like soils it is difficult to assess all possibilities of Cd binding in solid phase (quantity factor) as well as Cd in soil solution (intensity factor). Furthermore, the result indicates that the sequential extraction, although operationally defined, cannot provide valuable data for predicting the Cd availability to plants through the soil solution only in whether the plants can affect the fractions. Further study is required to develop more accurate speciation analysis methods so that well-defined species in soil solution can be determined convincingly.

5 SUMMARY

Plant species differ in Cd uptake from soil and accumulation in the shoot and therefore the objective of this research was to study to which extent those differences are based on the size of root system, on the Cd influx, on the growth rate of the shoot and on the proportion of the Cd taken up that is translocated to the shoot. It was also considered the influence of plants on Cd availability or solubility in soil and its correlation to Cd uptake by the plants. For this, maize, sunflower, flax, and spinach were grown on a sandy soil ($\text{pH}_{\text{CaCl}_2}$ 4.5, 2.8% C, 5% clay) with three levels of Cd addition (0, 14, and 40 $\mu\text{mol kg}^{-1}$ soil). Shoot growth, Cd concentration, and root growth were determined as well as the Cd soil solution concentration that was collected through a displacement method.

Shoot growth, shoot Cd accumulation and Cd net influx

At the low Cd addition of 14 $\mu\text{mol kg}^{-1}$ there was small growth stimulation for maize, sunflower and flax. Of the 4 plants screened, flax and spinach showed severe Cd toxicity symptoms at 40 $\mu\text{mol Cd kg}^{-1}$ and thus, were considered to be sensitive to Cd toxicity in comparison to maize and sunflower. Maize plants grown at 14 Cd $\mu\text{mol kg}^{-1}$ were approximately similar than those grown at 40 Cd $\mu\text{mol kg}^{-1}$, even though their shoot Cd concentrations increased from 1.2 to 8 mg kg^{-1} SDW, respectively without limiting shoot growth. Sunflower showed shoot Cd concentration of 9.7-25.8 mg kg^{-1} SDW while for flax and spinach was up to 66.4 mg kg^{-1} and 105.1 mg Cd kg^{-1} , respectively.

The Cd shoot influx for sunflower and flax increased linearly with the Cd addition but for spinach and maize, the increase was more than proportional. For a 3-fold increase of Cd addition (14 to 40 $\mu\text{mol kg}^{-1}$) the Cd influx increased 8-fold (from 0.2×10^{-16} to 1.6×10^{-16} $\text{mol cm}^{-2} \text{s}^{-1}$) for maize and 6-fold (from 7.5×10^{-16} to 42×10^{-16} $\text{mol cm}^{-2} \text{s}^{-1}$) for spinach. The Cd shoot influx varied among species at the same Cd level by a factor of up to 37.

The shoot Cd concentration differed among species by a factor of up to 30. The root area-shoot dry weight ratio (RA / SDW) the highest and lowest value differed by a factor of two to three, spinach having the lowest value which would rather cause a lower Cd shoot concentration in contrast to what actually was found. The relative shoot growth rate (RGR_s) varied by a factor of maximum of 1.7. RGR_s of maize and sunflower was higher than in spinach and flax, irrespective of the level of Cd addition. The proportion of Cd translocated to shoot varied in the low (14 $\mu\text{mol Cd kg}^{-1}$) and high (40 $\mu\text{mol Cd kg}^{-1}$) addition from 60% to 70% in the less sensitive species and from 70% to 85% in the more sensitive. Spinach, the most sensitive species, always showed the highest translocation rate. However, the variation of 60% to 85% could not explain the variation of shoot Cd concentration by a factor of about 10 to 30. Similarly, the variation of the root-shoot ration and the relative shoot growth rate had less influence and could not explain the variation in shoot Cd concentration. It was mostly the Cd influx that varied by a factor of up to 30 which determined the different Cd concentration in the shoot.

Effect of plants on Cd concentration in soil solution and Cd influx

Only the Cd net influx varied parallel to the shoot Cd concentration, i.e. the Cd influx was the major factor responsible for the different Cd accumulation in the

shoot. Even though spinach had a smaller RA / SDW and a lower RGR_s, it achieved a higher shoot Cd concentration because it achieved a 26 times higher Cd influx than maize. The lower shoot Cd concentration of maize was attributed to the lower influx. Cadmium influx depends on the uptake kinetics expressed by the root absorbing power, α , which is the slope of the almost linear portion of the uptake isotherm in the lower concentration range and the effect of the root on Cd activity (i.e. the effective Cd concentration) in the soil solution. Plant growth changed Cd concentration in soil solution. The Cd concentration in soil solution significantly decreased after the growth of maize, sunflower, and spinach at the low Cd addition. For example, Cd concentration was decreased by about 65% after the growth of maize and by about 41% after the growth of sunflower compared to the control. After the growth of flax, the soil solution concentration of Cd was 84% higher than the control. At the high Cd addition ($40 \mu\text{mol Cd kg}^{-1}$) also spinach increased Cd soil solution concentration but much less than flax. This shows that plants affected Cd availability in soil, in some cases decreasing in other cases increasing C_{Li} i.e. immobilizing or solubilizing Cd. However, these changes in C_{Li} were not enough to explain differences in Cd influx. Shoot Cd influx was linearly related to Cd concentration in soil solution that was found after the plant had grown in the soil and for maize, sunflower and flax adjusted to about the same line with a slope of around $2.5 \times 10^{-7} \text{ cm s}^{-1}$ while spinach showed a clearly, 5 times, higher slope of $13.6 \times 10^{-7} \text{ cm s}^{-1}$.

Description of Cd influx by a deterministic model

Further explanations for the different Cd influx were sought by using mechanistic modeling of Cd uptake from soil comparing the simulated Cd influx with the measured Cd influx. The model NST 3.0 uses soil and plant parameters to calculate

as well as the Cd concentration distribution around the root, i.e. Cd dynamics in the rhizosphere. The model assumes that Cd transport to the root is by mass flow and diffusion and that the uptake into the root follows Michaelis-Menten kinetics. For Cd we used the root absorbing power, α , which approximates the uptake isotherm in the low concentration range. These calculations will help to understand why plants differ in their Cd accumulation. The model over-predicted Cd influx in all plants. For maize, it was up to 10 times and for flax 7 times, for sunflower 4.7 times whereas for spinach it was only 1.6. Therefore, sensitivity analysis for α , and C_{Li} was conducted in order to find which parameter might have been wrongly estimated or influenced by plants in the rhizosphere. In the sensitivity analysis, stepwise-reduced values of C_{Li} and α were used.

The sensitivity analysis for C_{Li} showed that by decreasing the value down to 10% of the original value got 100% prediction of measured Cd influx of maize and the C_{Li} decreased only by $0.019\mu\text{M}$. For spinach, the same was achieved by decreasing C_{Li} down to 63% of the original value and according to the model calculations, decreased solution Cd concentration at the root surface to $0.22\mu\text{M}$ as compared to $0.019\mu\text{M}$ for maize after 14 days of uptake. This shows that the soil is able to transport Cd to the root surface but maize cannot achieve a higher influx at this lower Cd concentration in comparison to spinach.

On the other hand, when decreasing the root absorbing power (α value) of maize down to 10% of the original value caused an increased Cd concentration at the root surface from $0.22\mu\text{M}$ to $0.24\mu\text{M}$ because transport by mass flow exceeded Cd influx. In this case, limiting factor for Cd uptake was not soil Cd transport but uptake kinetics, i.e. α value. For spinach, calculated Cd influx was equal to measured Cd influx after decreasing the root absorbing power down to 54% but in

contrast to maize the Cd concentration at the root surface decreased from 0.48 μM to 0.46 μM . The root were able to decrease the concentration further ($C_L = 0.46 \mu\text{M}$), thereby creating a small concentration gradient to allow diffusive transport of Cd towards the root. The results of sensitivity analysis showed that the Cd uptake without consideration of other not well-defined processes, such as immobilization or complexation in soil solution (i.e. effective concentration), was not explained. The high degree of sensitivity to C_{Li} and α value for maize indicate that uptake kinetics (α value) was the major limiting factor for uptake and not Cd mobility through the soil towards the root. The amount of overestimation indicates the dimension of o possible complexation of Cd in soil solution. Maize and spinach have different uptake kinetics and they possible are able to change the composition of soil solution through root activity.

Plant growth on Cd binding in soil

Plants were able to solubilized or immobilized Cd in soil because they changed Cd solution concentration resulting in maize and spinach to reduce and flax to increase it. This change will affect the equilibrium of Cd in soil solution with Cd fractions in soil or its distribution, and its chemical form in the soil. This was investigated in the soil enriched with 40 $\mu\text{mol Cd kg}^{-1}$ by a fractionation of soil Cd with a sequential extraction. The seven binding fractions of Cd are operationally defined by an extraction sequence that follows the order of increasing acidity with extractants of different complexing and redox properties. These fractions are: mobile fraction (F.I), easily mobilizable fraction (F.II), occluded in Mn oxides bound fraction(F.III), organically bound fraction (F.IV), occluded in poorly crystalline Fe oxide bound fraction (F.V), occluded in crystalline Fe oxides bound fraction (F.VI) and residual fraction (F.VII).

In soil without plants, Cd was mostly concentrated in F.I (14.3 $\mu\text{mol Cd kg}^{-1}$) and F.II (13.1 $\mu\text{mol Cd kg}^{-1}$) which reflects the greater tendency of Cd to bind as exchangeable in this soil, although it was also present in other fractions. The Cd bound in F.III was 9.6 $\mu\text{mol Cd kg}^{-1}$ (22%) and Cd bound to organic matter in F.IV was lower (5 $\mu\text{mol kg}^{-1}$ or 10%). The remaining fractions represented less than 5%.

Fraction I (mobile fraction): all plants decreased the content of this fraction. This decrease of 1.6 $\mu\text{mol kg}^{-1}$ to 2.7 $\mu\text{mol kg}^{-1}$ was more than Cd uptake, which varied from 0.5 $\mu\text{mol kg}^{-1}$ to 2.2 $\mu\text{mol kg}^{-1}$.

Fraction II (easily mobilizable): In F.II recorded a negative change of Cd in the soil grown to flax (1.7 $\mu\text{mol kg}^{-1}$) whereas in maize (3.5 $\mu\text{mol kg}^{-1}$) and spinach (4 $\mu\text{mol kg}^{-1}$) is noticed a positive change indicating a change in the Cd distribution, in the soil due to plant growth.

Fraction III (occluded in Mn oxides): There was a significant reduction in sunflower (3.7 $\mu\text{mol kg}^{-1}$) and a little in spinach (2.5 $\mu\text{mol kg}^{-1}$). F.III could have covered Cd uptake of sunflower (0.8 $\mu\text{mol kg}^{-1}$) and spinach (2.2 $\mu\text{mol kg}^{-1}$). The negative change in the maize (0.6 $\mu\text{mol kg}^{-1}$) was almost similar to the Cd uptake. The Cd content in flax was not change and had no effect on Cd uptake.

Fraction IV (organically bound fraction): In F.IV the Cd content increased clearly in maize and flax (1.7 $\mu\text{mol Cd kg}^{-1}$) while in spinach the increase was very low and sunflower had no effect.

This shows that plants change the binding of Cd in soil in a different manner, depending on the plants species. Plants also changed Cd in soil solution; it varied from 0.5 μM with maize to 5.8 μM with flax. The fact that the Cd concentration in

soil solution decreased (Maize, Sunflower), but on the other increased (spinach, flax) might indicate different a distribution of Cd fractions in soil. However, there was no relationship between changes in Cd fraction and changes of Cd concentration in soil solution. Also only insufficient information exists about the availability of organic Cd complexes in the solution phase. Under the assumption that Cd is present in soil solution complexed or chelated, then the effective Cd soil solution concentration would be much lower. Based on the results from the sensitivity analysis, we assume a very low value of Cd concentration (i.e. effective Cd concentration) and/or a different α value.

6 ZUSAMMENFASSUNG

Pflanzenarten unterscheiden sich in der Cd-Aufnahme aus dem Boden und in der Cd-Akkumulation im Spross und somit das Ziel dieser Studie war zu untersuchen, inwieweit diese Unterschiede auf der Größe des Wurzelsystems, auf dem Cd-Influx, auf der Wachstumsrate des Sprosses und/oder der Translokation des Cd von der Wurzel in den Spross basieren. Es wurde auch der Einfluss von Pflanzen auf die Cd-Verfügbarkeit bzw. die Löslichkeit im Boden und ihre Beziehung zur Cd-Aufnahme durch die Pflanzen betrachtet. Für diesen Vorhaben wurde Mais, Sonnenblumen, Öllein, und Spinat auf einem sandigen Boden (pH CaCl_2 4,5, 2,8% C, 5% Ton) mit drei Cd-Stufen (0, 14 und 40 $\mu\text{mol kg}^{-1}$ Boden) angebaut. Spross- und Wurzelwachstum und Cd-Gehalte wurden bestimmt sowie die Cd-Konzentration in der Bodenlösung, die durch eine Verdrängung gewonnen wurde

Sprosswachstum, Cd-Akkumulation im Spross und Cd-Influx

Bei der niedrigen Cd-Zufuhr von 14 $\mu\text{mol kg}^{-1}$ gab es eine geringe Wachstumsstimulation für Mais, Sonnenblumen und Öllein. Von den 4 untersuchten Pflanzen, zeigten Öllein und Spinat schwere Cd-Toxizitätssymptome bei 40 $\mu\text{mol Cd kg}^{-1}$ und waren somit empfindlicher gegenüber Cd-Toxizität als Mais und Sonnenblume. Das Wachstum von Mais auf 14 Cd $\mu\text{mol kg}^{-1}$ war ähnlich mit dem bei 40 Cd $\mu\text{mol kg}^{-1}$, obwohl die Cd-Konzentration im Spross von 1,2 mg kg^{-1} auf 8 mg kg^{-1} TM erhöht wurde. Bei Sonnenblume erhöhte sich die Cd-Konzentration im Spross von 9,7 mg kg^{-1} bis 25,8 mg kg^{-1} TM während sie für Öllein und Spinat auf 66,4 mg kg^{-1} bzw. 105,1 mg kg^{-1} TM anstieg.

Der Cd-Sprossinflux von Sonnenblume und Öllein erhöhte sich linear mit steigender Cd-Zufuhr aber für Spinat und Mais war der Anstieg überproportional. Für eine 3-fach erhöhte Cd-Zufuhr (von $14 \mu\text{mol kg}^{-1}$ auf $40 \mu\text{mol kg}^{-1}$) im Boden stieg der Cd-Sprossinflux bei Mais 8-fach (von $0,2 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$ auf $1,6 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$) und 6-fach (von $7,5 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$ auf $42 \times 10^{-16} \text{ mol cm}^{-2} \text{ s}^{-1}$) für Spinat an. Der Cd-Sprossinflux variierte zwischen den Pflanzenarten innerhalb derselben Cd-Stufe um einen Faktor von bis zu 37.

Die Cd-Konzentration im Spross variierte zwischen den Pflanzenarten um einen Faktor von bis zu 30. Das Wurzeloberfläche/Spross-Verhältnis (WSV) variierte um den Faktor zwischen 2 und 3, wobei Spinat den niedrigsten Wert hatte. Ein niedriger Wert würde eher zu einer niedrigeren Cd-Konzentration im Spross führen, aber stattdessen war der Wert größer. Die relative Wachstumsrate des Sprosses (RGR_S) variierte um einen Faktor von maximal 1,7. RGR_S von Mais und Sonnenblumen war höher als in Spinat und Öllein, unabhängig von der Höhe der Cd-Zufuhr. Der translozierte Cd-Anteil im Spross variierte zwischen der niedrigen ($14 \mu\text{mol Cd kg}^{-1}$) und der höchsten Cd-Zufuhr ($40 \mu\text{mol Cd kg}^{-1}$) um 60% - 70% bei den weniger empfindlichen Pflanzenarten und um 70% - 85% bei den empfindlichen Pflanzenarten. Spinat, der empfindlichsten Pflanzenart, zeigte immer die höchste Translokationsrate. Die Variation von 60% bis 85% könnte allerdings nicht den Unterschied in der Cd-Konzentration im Spross bei einem Faktor von etwa 10 bis 30 erklären. Ebenso hatte die Variation des Wurzeloberfläche/Spross Verhältnisses und die relative Wachstumsrate des Sprosses weniger Einfluss und konnte nicht die Variation der Cd-Konzentration im Spross erklären. Es war vor allem der Cd-Sprossinflux, der durch einen Faktor von bis zu 30 variierte, und bestimmte damit die Cd-Konzentration im Spross

Wirkung von Pflanzen auf die Cd-Konzentration in der Bodenlösung und auf den Cd-Sprossinflux

Nur der Cd-Sprossinflux variierte parallel zu der Cd-Konzentration im Spross, d.h. der Cd-Sprossinflux war der Hauptfaktor für die unterschiedliche Cd-Akkumulation im Spross. Obwohl Spinat ein kleineres WSV und eine niedrige RGR_s hatte, erzielte er eine höhere Cd-Konzentration im Spross, weil er einen 26-mal höheren Cd-Sprossinflux als Mais hatte. Die niedrigere Cd-Konzentration im Spross von Mais war auf den niedrigeren Cd-Sprossinflux zurückzuführen. Cadmium-Sprossinflux hängt ab von der Aufnahmekinetik ausgedrückt durch die Wurzel-Aufnahmefähigkeit (root absorbing power), α , die das Steigungsmaß des fast linearen Teils der Aufnahme-Isotherme im unteren Konzentrationsbereich beschreibt und von der Wurzelwirkung auf die Cd-Aktivität (d.h. die effektive Cd-Konzentration) in der Bodenlösung. Das Pflanzenwachstum beeinflusste die Cd-Konzentration in der Bodenlösung. Die Cd-Konzentration in der Bodenlösung nahm deutlich nach dem Wachstum von Mais, Sonnenblume, und Spinat bei der niedrigen Cd-Zufuhr ab. Zum Beispiel wurde die Cd-Konzentration in der Bodenlösung (C_{Li}) durch Mais um etwa 65% und durch Sonnenblume um 41% gegenüber den Kontrolltöpfen erniedrigt. Dagegen war nach Wachstum von Öllein, die C_{Li} um 84% erhöht. Bei der hohen Cd-Zufuhr ($40 \mu\text{mol Cd kg}^{-1}$) waren auch bei Spinat C_{Li} erhöht aber deutlich weniger als bei Öllein. Dies zeigt, dass die Pflanzen die Cd-Verfügbarkeit im Boden beeinflussten, in einigen Fällen erniedrigten sie in anderen Fällen erhöhten sie C_{Li} , d.h. die Pflanzen immobilisierten bzw. lösten das Cd im Boden. Diese Änderungen von C_{Li} waren allerdings nicht genug, um die Unterschiede im Cd-Sprossinflux zu erklären. Der Cd-Sprossinflux war linear korreliert mit der Cd-Konzentration in der Bodenlösung, die gefunden

wurde, nachdem die Pflanzen im Boden gewachsen waren. Für Mais, Sonnenblumen und Öllein war das Steigungsmaß dieser linearen Beziehung ähnlich hoch, von rund $2,5 \times 10^{-7} \text{ cm s}^{-1}$, während bei Spinat war sie mit $13,6 \times 10^{-7} \text{ cm s}^{-1}$ um den Faktor 5 deutlich höher.

Beschreibung von Cd-Sprossinflux durch ein deterministisches Modell

Weitere Erklärungen für den unterschiedlichen Cd-Sprossinflux wurden mit Hilfe von mechanistischer Modellierung den Cd-Aufnahme aus dem Boden gesucht, indem der simulierte Cd-Sprossinflux mit dem gemessenen Cd-Sprossinflux verglichen wurde. Das Modell NST 3.0 verwendet Boden- und Pflanzenparameter um sowohl den Cd-Sprossinflux zu berechnen als auch die Cd-Konzentrationsverteilung um die Wurzel, d.h. Cd-Dynamik in der Rhizosphäre zu verzeichnen. Das Modell setzt voraus, dass Cd-Transport an die Wurzel durch Massenfluss und Diffusion erfolgt und dass die Aufnahme in die Wurzel mit der Michaelis-Menten-Kinetik beschrieben wird. Für Cd wurde die Wurzel-Aufnahmefähigkeit (root absorbing power), α , verwendet, welche sich die Aufnahme-Isotherme im niedrigen Konzentrationsbereich annähert. Diese Berechnungen könnten zusätzliche Information liefern, warum die Pflanzen sich in ihrer Cd-Akkumulation im Spross unterschieden. Das Modell überschätzte den Cd-Sprossinflux bei allen Pflanzenarten. Bei Mais war es bis zu 10-mal, für Öllein 7-mal und für Sonnenblumen 4,7-mal während es für Spinat nur 1,6 war. Daher wurde eine Sensitivitätsanalyse für α und C_{Li} durchgeführt, um herauszufinden, welche von den eingegebenen Parametern es gewesen sein könnten, die falsch eingeschätzt oder von den Pflanzen in der Rhizosphäre beeinflusst waren. In der Sensitivitätsanalyse wurden stufenweise die Werte von C_{Li} und α gesenkt. Die Sensitivitätsanalyse zeigte, dass C_{Li} bei Mais bis auf 10% und bei Spinat bis auf 63%

des ursprünglichen Wertes gesenkt werden musste um eine 100% Übereinstimmung des gemessenen mit dem berechneten Cd-Sprossinflux zu erreichen.

Auf der anderen Seite, wenn die Wurzel-Aufnahmefähigkeit (α -Wert) von Mais auf 10% des ursprünglichen Wertes gesenkt wurde, verursachte es eine Erhöhung der Cd-Konzentration an der Wurzeloberfläche von 0,22 μM auf 0,24 μM , da der Cd-Transport durch Massenfluss den Cd-Sprossinflux überstieg. In diesem wie auch in den anderen Fällen, war der limitierende Faktor für die Cd-Aufnahme nicht der Cd-Transport im Boden sondern die Cd-Aufnahmekinetik, d.h. der α -Wert. Für Spinat, der berechnete Cd-Sprossinflux war gleich den gemessenen Cd-Sprossinflux nach Verringerung von α -Wert auf 54%, aber im Gegensatz zu Mais die Cd-Konzentration an der Wurzeloberfläche sank von 0,48 μM auf 0,46 μM . Die Wurzeln konnten die Konzentration weiter verringern ($C_L = 0,46 \mu\text{M}$), wodurch ein kleiner Konzentrationsgradient zu diffusiven Cd-Transport in Richtung der Wurzel entstand. Die Ergebnisse der Sensitivitätsanalyse zeigten, dass die Cd-Aufnahme ohne Berücksichtigung anderer nicht gut definierte Prozesse, wie z.B. Immobilisierung oder Komplexierung in der Bodenlösung (d.h. effektive Cd-Konzentration) nicht erklärt werden konnten. Die hohe Empfindlichkeit auf C_{Li} und α -Wert für Mais wiesen darauf hin, dass die Aufnahmekinetik (α -Wert) der limitierende Faktor für die Cd-Aufnahme und nicht die Cd-Mobilität im Boden war. Die Höhe der Überschätzung deutete die Dimension einer möglichen Komplexierung von Cd in der Bodenlösung an. Mais und Spinat haben unterschiedliche Aufnahmekinetik und sie waren möglicherweise in der Lage, die Zusammensetzung der Bodenlösung durch Wurzelaktivität zu verändern.

Einfluss des Pflanzenwachstums auf die Cd-Bindung im Boden

Pflanzen konnten Cd im Boden lösen oder immobilisieren, weil Mais und Spinat die Cd-Konzentration in der Bodenlösung reduziert haben hingegen Öllein sie erhöht hat. Diese Veränderung könnte auf das Gleichgewicht von Cd in der Bodenlösung mit den Cd-Fractionen im Boden oder dessen Verteilung und die chemische Form im Boden zurückgeführt werden. Dies wurde im Boden mit $40 \mu\text{mol Cd kg}^{-1}$ durch eine Fraktionierung mit einer sequentiellen Cd-Extraktion untersucht. Die sieben Cd-Fractionen sind operativ durch eine Extraktionsfolge definiert, da die Extraktionsmittel zunehmende Acidität und/oder unterschiedliche Komplexierungs- und Redoxeeigenschaften aufweisen. Diese Fractionen sind: mobile Fraction (F.I), leicht mobilisierbare Fraction (F.II), in Mn-Oxiden okkludierte Fraction (F.III), organisch gebundene Fraction (F.IV), in schlecht kristallinen Fe-Oxiden okkludiert Fraction (F.V), in kristallinen Fe-Oxide okkludiert Fraction (F.VI) und Residualfraction (F. VII).

In Bodenproben ohne Pflanzen, Cd war meist in F.I ($14,3 \mu\text{mol Cd kg}^{-1}$) und F. II ($13,1 \mu\text{mol Cd kg}^{-1}$) konzentriert, dass die größeren Neigung von Cd reflektiert als austauschbar im Boden gebunden zu sein, wobei es auch in anderen Fractionen vorhanden war. Der Cd-Anteil in F.III betrug $9,6 \mu\text{mol Cd kg}^{-1}$ (22%) und in F. IV war niedriger ($5 \mu\text{mol kg}^{-1}$ oder 10%). Auf die übrigen Fractionen entfielen weniger als 5%.

Fraktion I (mobile Fraction): alle Pflanzen senkten den Cd-Gehalt dieser Fraction. Dieser Rückgang von $1,6 \mu\text{mol kg}^{-1}$ bis $2,7 \mu\text{mol kg}^{-1}$ war mehr als die Cd-Aufnahme, die von $0,5 \mu\text{mol kg}^{-1}$ bis $2,2 \mu\text{mol kg}^{-1}$ variierte.

Fraktion II (leicht mobilisierbar): In F.II verzeichnete eine negative Veränderung von Cd-Gehalt in Bodenproben mit Öllein ($1,7 \mu\text{mol kg}^{-1}$) während die mit Mais ($3,5 \mu\text{mol kg}^{-1}$) und Spinat ($4 \mu\text{mol kg}^{-1}$) eine positive Veränderung zeigte. Dies zeigt eine Veränderung in der Cd-Verteilung im Boden durch das Pflanzenwachstum an.

Fraktion III (okkludiert in Mn-Oxide): Es gab eine signifikante Reduktion von Cd-Gehalt bei Sonnenblume ($3,7 \mu\text{mol kg}^{-1}$) und ein wenig bei Spinat ($2,5 \mu\text{mol kg}^{-1}$). F.III könnte die Cd-Aufnahme von Sonnenblumen ($0,8 \mu\text{mol kg}^{-1}$) und Spinat ($2,2 \mu\text{mol kg}^{-1}$) gedeckt haben. Die negative Veränderung bei Mais ($0,6 \mu\text{mol kg}^{-1}$) war fast gleich wie die Cd-Aufnahme. Der Cd-Gehalt bei Öllein war nicht verändert und könnte keinen Einfluss auf Cd-Aufnahme haben.

Fraktion IV (organisch gebundene Fraktion): In F. IV der Cd-Gehalt stieg deutlich bei Mais und Öllein ($1,7 \mu\text{mol Cd kg}^{-1}$) an, während bei Spinat der Anstieg sehr gering war und bei Sonnenblumen keine Wirkung hatte.

Dies zeigt, dass Pflanzen die Cd-Bindung im Boden in unterschiedlicher Weise, je nach Pflanzenart verändern können. Pflanzen haben auch die Cd-Konzentration in der Bodenlösung verändert, wobei die Variation von $0,5 \mu\text{M}$ bei Mais bis auf $5,8 \mu\text{M}$ bei Öllein schwankte. Die Tatsache, dass die Cd-Konzentration in der Bodenlösung (Mais, Sonnenblumen) abgenommen, aber auf der anderen erhöht (Spinat, Öllein) wurde, könnte auf eine unterschiedliche Verteilung von Cd-Fraktionen im Boden hindeuten. Es gab jedoch keinen Zusammenhang zwischen Veränderungen in der Cd-Fraktion und Veränderungen der Cd-Konzentration in der Bodenlösung. Ebenfalls nur unzureichende Informationen liegen über die Verfügbarkeit von organischen Cd-Komplexen in der Lösungsphase vor. Unter der

Annahme, dass Cd in der Bodenlösung komplexiert oder chelatisiert wäre, dann wäre die effektive Cd-Konzentration in der Bodenlösung viel niedrigere. Basierend auf den Ergebnissen der Sensitivitätsanalyse, nehmen wir einen sehr niedrigen Wert der Cd-Konzentration in der Bodenlösung (d.h. effektive Cd-Konzentration) und / oder einen niedrigeren α -Wert an.

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8 APPENDIX

Table 8-1 The sequential extraction scheme for Cd fractionation after Zeien and Brümmer (1989)

Fraction	Extractant	Extraction
I.	1M NH ₄ NO ₃ (unbuffered)	24 h shaking
II.	1 st step 1 M NH ₄ OAc (pH 6.0) 2 nd step 1M NH ₄ NO ₃ (unbuffered)	1 st step 24 h shaking 2 nd step 10 min
III.	1 st step 0.1 M NH ₂ OH-HCl + 1M NH ₄ OAc (pH 6.0) 2 nd step (2 times) 1 M NH ₄ OAc (pH 6.0)	1 st step 30 min shaking 2 nd step 10 min (2 times)
IV.	1 st step 0.025 M NH ₄ -EDTA +1 M NH ₄ OAc (pH 4.6) 2 nd step 1 M NH ₄ OAc (pH 4.6)	1 st step 90 min shaking 2 nd step 10 min
V.	1 st step 0.2 M NH ₄ -oxalate-buffer (pH 3.25) 2 nd step 0.2 M NH ₄ -oxalate-buffer (pH 3.25)	1 st step 4 h shaking (indarkness) 2 nd step 10 min shaking (indarkness)
VI.	1 st step 0.1 M ascorbic acid + 0.2 M oxalate puffer (pH 3.25) 2 nd step 0.2 M NH ₄ -oxalate-buffer (pH 3.25)	1 st step 1h boiling at 96°C 2 nd step 10 min shaking (indarkness)
VII.	conc. HCl / conc. HNO ₃	45 min at 70 °C 30 min at 110 °C 180 min at 120 °C
Total Cd	HNO ₃	12 h at 180°C

Table 8-2 Partitioning of Cd in sandy soil (pH_{CaCl2} 4.5, 2.8%C, 5% clay) among the seven fractions of the sequential extraction of Zeien and Brümmer (1989) after addition of 40 $\mu\text{mol Cd kg}^{-1}$ soil

Fraction	Control	Maize	Sunflower	Flax	Spinach
----- $\mu\text{mol Cd kg}^{-1}$					
I.	14.3	12.8	11.7	11.8	11.7
II.	13.1	16.5	13.6	11.4	17.2
III.	9.6	9.0	5.9	9.6	7.1
IV.	4.3	6.1	4.3	6.0	4.8
V.	1.0	1.3	1.2	1.2	1.1
VI.	0.6	0.4	0.3	0.5	0.4
VII.	0.2	0.1	0.1	0.1	0.2
SUM	43.0	46.2	37.1	40.5	42.4
Cd _t -Teflon*	50.6	49.7	39.8	47.6	44.5
Retrieval (%)	85	93	93	85	95

*Determination of the total Cd (Cd_t) content was performed independently on another soil sample (0.2 g dry wt) in teflon box under high pressure for 12 h at 180°C.

Table 8-3 Soil solution Cd concentrations at first and second harvest obtained from modified displacement method according to Adams (1974)

Plant	C_{Li} $10^{-9} \text{ mol cm}^{-3}$		
	1 st Harvest	2 nd Harvest	average
----- 14 $\mu\text{mol Cd kg}^{-1}$			
Maize	0.3	0.2	0.22
Sunflower	0.5	0.3	0.38
Flax	0.9	1.5	1.19
Spinach	0.3	0.6	0.48
----- 40 $\mu\text{mol Cd kg}^{-1}$			
Maize	1.0	0.4	0.74
Sunflower	2.2	1.4	1.80
Flax	3.3	5.8	4.59
Spinach	1.9	4.3	3.07

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10 CURRICULUM VITAE

Name Christos Stritsis
Sex Male
Date of birth 19.11.1969
Place of birth Goumenissa, D. Paionias, P. Central Makedonia – Grece
Nationality Greek

Work Experiences

2007 – 2011 Research assistant in project “**Differential shoot Cd accumulation in crops**” at the Department of Crop Sciences, Institute of Agricultural Chemistry, Section of Plant Nutrition and Crop Physiology, University of Göttingen

2005 – 2007 Research/Teaching assistant at the Department of Crop Sciences, Institute of Agricultural Chemistry, Section of Plant Nutrition and Crop Physiology, University of Göttingen.

Education

2007 – 2010 **Ph.D.** Department of Crop Sciences, Section of Plant Nutrition and Crop Physiology, University of Göttingen

2003 – 2005 **M. Sc.** Department of Crop Sciences, Section of Plant Nutrition and Crop Physiology, University of Göttingen

1999 – 2003 **B. Sc.** Department of Crop Sciences, Section of Agricultural Entomology University of Göttingen

1981 – 1987 Gymnasium und Lyzeum in Griechenland

International Seminar

26 – 28.9. 2006 Symposium "Plant Nutrition meets Plant Breeding" in Hohenheim, Deutschland.
Topic: Differential cadmium accumulation in crops as related to their uptake kinetics and rhizosphere effects.

8 – 21.9. 2007 119. VDLUFA-Congress in Göttingen, Deutschland
Topic: Cadmium accumulation of maize, sunflower, spinach and flax as related to their uptake kinetics and rhizosphere effects.