



Självständigt arbete vid LTJ-fakulteten, SLU  
Master project in the Horticultural Science Program, 2009  
30 ECTS

## Effect of liming and free $\text{Ca}^{2+}$ on Cd uptake of carrots, *Daucus carota*



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Titel:  
**Effect of liming and free Ca<sup>2+</sup> on Cd uptake of carrots, *Daucus carota***

Svensk titel:  
**Effekten av kalkning och fria Ca<sup>2+</sup> på upptaget av Cd i morötter, *Daucus carota***

Program/Utbildning:  
**Hortonomprogrammet**

Examen:  
**Hortonomexamen**

Huvudområde:  
**Biologi**

Nyckelord:  
**Cd, Ca, Cd uptake, carrots, substrate, nutrient solution**

Handledare:  
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Kurskod:  
**EX0453**

Kurstitel:  
**Examensarbete inom hortonomprogrammet**

Omfattning (hp):  
**30**

Nivå och fördjupning:  
**E**

Utgivningsort:  
**Alnarp**

Månad, År:  
**Oktober, 2009**

Serie:  
**Självständigt arbete vid LTJ-fakulteten**

Omslagsfoto:  
**Anna Holmkvist**



Acknowledgments .....	7
Abstract .....	9
Sammanfattning .....	11
Introduction .....	13
Cadmium in our food .....	13
Effects of cadmium in the body .....	14
Risk groups.....	15
Cadmium in soils.....	15
Cadmium availability in soils.....	17
Uptake by plants.....	18
How to prevent/decrease Cadmium uptake.....	19
Aim.....	20
Materials and methods .....	20
Plant material and growth conditions.....	20
Long-term substrate experiment.....	21
Soil analyses.....	22
Short-term nutrient solution experiment .....	22
Results .....	25
Long-term soil experiment .....	25
Plant growth .....	25
Cadmium concentrations in plants .....	27
Calcium concentrations in plants .....	28
DTPA extraction .....	30
Short-term nutrient solution experiment .....	30
Discussion .....	32
Long-term soil experiment .....	32
Plant growth .....	32
Cd uptake and availability.....	33
Short-term nutrient solution experiment .....	34
Concluding remarks .....	35
References .....	37



## **Acknowledgments**

I would like to thank my supervisors Associate Professor Håkan Asp and PhD Heléne Larsson-Jönsson for their support and guidance as well as my examiner PhD Siri Caspersen. A special thanks to Partnerskap Alnarp for financially supporting my project and to Erik Kohlström for proofreading and the illustration over Cd fluxes. Last but not least I want to thank my friends who always offer me patience and support.





## Abstract

The level of cadmium (Cd) in Swedish arable land has increased during the last century and the average concentration of Cd in the plough layer is today 0.23 mg/kg. The increase is mainly due to anthropogenic activities such as phosphorus fertilizing, liming, import of feed, and atmospheric deposition from industrial emissions. Cadmium in the soil will be taken up by crops and is thereby transferred to humans via food. Carrot is one of the crops of concern, both because it may contain quite high Cd concentrations and due to the large consumption in Sweden.

In the body, Cd is stored mainly in the liver and the kidneys and may cause several diseases already at low concentrations. Due to these findings, the recommendation for Cd intake has recently been lowered and should not exceed 2.5 µg per kg body weight/ week. This means 25 µg per day for a person weighing 70 kg. To decrease the Cd uptake by plants liming is often recommended, however the results of liming are inconsistent. When adding lime several soil factors, such as pH, Ca concentration, and ion strength is altered which may affect the Cd availability and uptake by plants.

The aim of this Master thesis was to study the effects of liming and  $\text{Ca}^{2+}$  on the Cd uptake by carrots. In order to do that, two experiments were conducted. The long-term experiment was conducted in a climate chamber where the carrots were grown in a sand/peat substrate for eleven weeks. Two levels of Cd were tested, 0.23 and 2.3 mg/kg soil, in combination with three pHs: 4.6, 5.6, and 6.6. The pH was altered by addition of lime. In the short-term experiments the Cd uptake by plants was tested in nutrient solutions. Two different pH levels were used, 5.6 and 6.6. Each pH was combined with three levels of Ca, 2.25, 4.5, and 9 mM.

The results from the long term experiment showed increased Cd uptake with increasing pH. However, since the concentrations of Cd and Ca follow each other in all treatments and were well correlated to the plant weights it seems like the Cd uptake was mostly affected by plant growth. Results from the short-term experiment showed decreased Cd uptake with increasing pH. The results also indicate that high Ca concentrations may lower the Cd uptake. However the effect of Ca seems only to be valid at low pH.



## Sammanfattning

Halten av kadmium (Cd) i Svensk åkermark har ökat under det senaste århundradet och medelvärdet i matjorden är idag 0.23 mg/kg jord. Ökningen kommer främst från antropogena källor som till exempel användandet av fosforgödsel och kalk, import av foder samt atmosfäriskt nedfall från industriutsläpp. Kadmium i jorden tas upp av grödorna och överförs på så sätt till människan via maten. Morötters upptag av Cd är av intresse dels för att de kan innehålla ganska höga halter av Cd, men också för att vi konsumerar mycket morötter i Sverige.

När Cd tagits upp i kroppen lagras det i levern och njurarna. Nya studier har visat att redan låga halter Cd i kroppen kan bidra till olika sjukdomar som benskörhet och livmoderhalscancer. På grund av dessa nya indikationer har rekommendationen för maximalt intag av Cd sänkts till 2.5 µg per kg kroppsvikt och vecka. Det motsvarar ungefär 25 µg per dag för en person som väger 70 kg. En lägre koncentration av Cd i grödorna skulle leda till lägre Cd intag via maten. Kalkning är den vanligaste åtgärden som rekommenderas för att sänka växternas upptag av Cd, dock ger kalkning inkonsekventa resultat på Cd upptag i flera studier. När man kalkar ändrar man flera jordfaktorer som kan påverka tillgängligheten och upptaget av Cd, till exempel pH, kalciumkoncentration (Ca) och jonstyrka.

Målet med detta Masterprojektet var att undersöka vilken effekt kalkning och  $\text{Ca}^{2+}$  har på Cd-upptaget i morötter. Inom projektet utfördes två experiment. Det ena var ett långtidsförsök där morötter odlades i ett sand/torv-substrat under elva veckor. Studien genomfördes med två nivåer av Cd, samt tre nivåer av kalk. Det andra försöket var ett korttidsförsök där Cd-upptaget i näringslösning innehållande Cd testades. Två olika pH-värden i kombination med tre olika Ca-koncentrationer undersöktes.

Resultaten från långtidsförsöket visar ökat Cd-upptag med ökande pH. Då koncentrationen av Cd och Ca följde varandra i alla behandlingar samt var väl korrelerade med plantornas vikt, är det troligt att tillväxten var den viktigaste faktorn som påverkade Cd-upptaget i försöket. Korttidsförsöket gav minskat Cd-upptag med ökande pH. Resultaten indikerar även att hög Ca-koncentration kan minska Cd-upptaget, men effekten verkar bara gälla för lågt pH.



## **Introduction**

Cadmium (Cd) levels in arable land have increased during the last century due to anthropogenic activities. Most of the Cd transferred to humans comes from food, especially plant food. Carrots are of particular concern due to a large consumption of this vegetable. In the body, Cd is stored and may disturb essential functions and cause diseases and organ failure.

To limit the intake of Cd by food, plant uptake is a good starting point. Soil properties and the cultivating measures greatly influence the Cd uptake by plants and are therefore of great concern. Liming has been suggested as a way to decrease plant Cd uptake but the results are inconsistent.

### ***Cadmium in our food***

For non-smokers the major Cd exposure, 90%, comes from food (EFSA, 2009). Plant food contributes to more than 80% of the dietary cadmium intake. Mainly cereals, vegetables, root vegetables and potatoes are of concern. This is not because these products have particularly high Cd levels. It is rather the fact that we consume large amounts of these commodities. Among products with high Cd levels we find linseed, sunflower seed, kidney and liver from mammals and hepatopancreas in shellfish (Jorhem and Sundström, 1993) fungi, legumes and nuts (EFSA, 2009). However, these products are normally not consumed in large amounts and are therefore not an equally big problem.

The concentration of Cd is of course important, but one also has to consider how much is consumed of different products and how often. It is a matter of both Cd concentration and amount consumed. Cereals for example, are a major part of the food intake in the Western world and because of this their Cd levels are of concern.

Carrot is one of the vegetables of concern, both because it contains quite high Cd concentrations and due to a large consumption. In Sweden the consumption of fresh carrots is about 8.3 kg/person and year (Eidstedt et al., 2004). The hygienic limit for Cd in root vegetables, such as carrots, within EU is 0.1 mg/kg fresh weight (Anonymous 2008). This corresponds to about 0.9 mg Cd/kg dry weight.

Today many cereal products contain wholegrain by reason of the fibres' positive effect on the health. This will contribute to a higher Cd intake via food since wholegrain contains higher concentration of Cd (EFSA, 2009). Today many people are aware of what they eat and try to have a healthy diet with wholegrain and a lot of vegetables. They probably have a higher intake of cadmium than others.

### ***Effects of cadmium in the body***

According to estimations 3 to 5% of the Cd in the food is absorbed during digestion (EFSA, 2009). In the body the absorbed Cd is bound to a protein (metallothionein) in the liver. Some of these complexes leach to the kidney where Cd is accumulated in the kidney cortex (Arbets- och miljömedicin, Uppsala, 2009). The highest concentration in the liver is reached at an age of 20 to 25 and in the kidney between 50 and 60 (EFSA, 2009). After that, the concentration slightly decreases.

The biological half life of Cd is very long, ranging from 10-30 years (EFSA, 2009). In the kidney, Cd causes damages on tubular cells and a level of 200 µg/g cortex is considered a critical level. Exceeding this concentration will cause irreversible damages and eventually lead to kidney failure. The damage of kidney cells will also affect the calcium- and phosphorus metabolism, which increases the risk for kidney stone and Osteogenesis Imperfecta (brittle-bone disease). That was what happened in Japan in the 1960s where, mostly women, who ate rice grown close to a mining company, got the itai-itai disease (Arbets- och miljömedicin, Uppsala, 2009). Activities in the mine were leaching Cd to the surroundings and this led to high crop Cd levels. When people consumed the crops, Cd accumulated in their bodies made their bones weak and easily broken. This caused a lot of pain, hence the name itai-itai, which means ouch-ouch in Japanese.

According to EFSA (2009) it is likely that Cd already at low levels contributes to Osteogenesis Imperfecta. It is also thought to disturb hormones, mainly oestrogen, in the body, and might cause cervical cancer after long time exposure (Åkesson et al., 2008). Due to these new indications the recommendation has recently been lowered. The recommendation for Cd is not more than 2.5 µg per kg body weight/ week (EFSA, 2009). This means 25 µg per day for a person weighing 70 kg.

## ***Risk groups***

Certain groups in the population are considered as risk groups for Cd exposure. Among those we find vegetarians, children, smokers and people living in contaminated areas (EFSA, 2009). Vegetarians are considered as a risk group since they have a higher intake of vegetables, which is one of the main sources of Cd in the diet. They also tend to consume more legumes, which often have high Cd concentrations, than the average population.

The exposure for children under 12 years is almost 60% higher than for adults (EFSA, 2009). This is due to the fact that children have greater food consumption in relation to body weight than adults do. Dust may also be an important source of exposure for children.

For smokers the Cd coming from their diet is of less concern since they already have a large Cd intake from cigarettes (EFSA, 2009). Tobacco leaves contain high concentrations of Cd. That, as well as the fact that lung cells are more prone to take up Cd than cells in the intestine, result in very high Cd levels in the bodies of smokers. They may have the double amount of Cd compared to non-smokers.

There is a confirmed relation between low iron (Fe) levels in the body and increased Cd absorption (EFSA, 2009). Women in fertile age are therefore a potential risk group in risk assessments since they tend to have lower Fe-status than men due to menstruation. Long periods of Fe-deficiency and multiple pregnancies may lead to increased levels of Cd stored in organs. Also low levels of zinc (Zn) and calcium (Ca) may result in an increased Cd accumulation. To restrict the accumulation it is therefore important to keep a good status of Fe, Zn and Ca.

## ***Cadmium in soils***

The levels of Cd in soils naturally fluctuate (Eriksson, 2009). The parent material has the biggest influence of the Cd content in a soil. Soils deriving from alum shale-rich parent material have among the highest levels of Cd. Because of alum shale's high level of sulphides a lot of heavy metals, there among Cd, were taken up during the formation of alum-shale millions of years ago.

Due to anthropogenic influences, the Cd levels in arable land have increased during the last century (Eriksson, 2009). The amount of Cd that is transported away from the fields by harvests and drainage water is less than what is added, and the result is increasing Cd levels in agricultural soils (Olsson et al., 2005). The increase during the 20<sup>th</sup> century was estimated by Eriksson (2009) to around 30% and today the mean value of Cd in the plough layer in Swedish agricultural soils is on average 0.23 mg/kg (extracted with HNO<sub>3</sub>) (Eriksson et al., 1997).

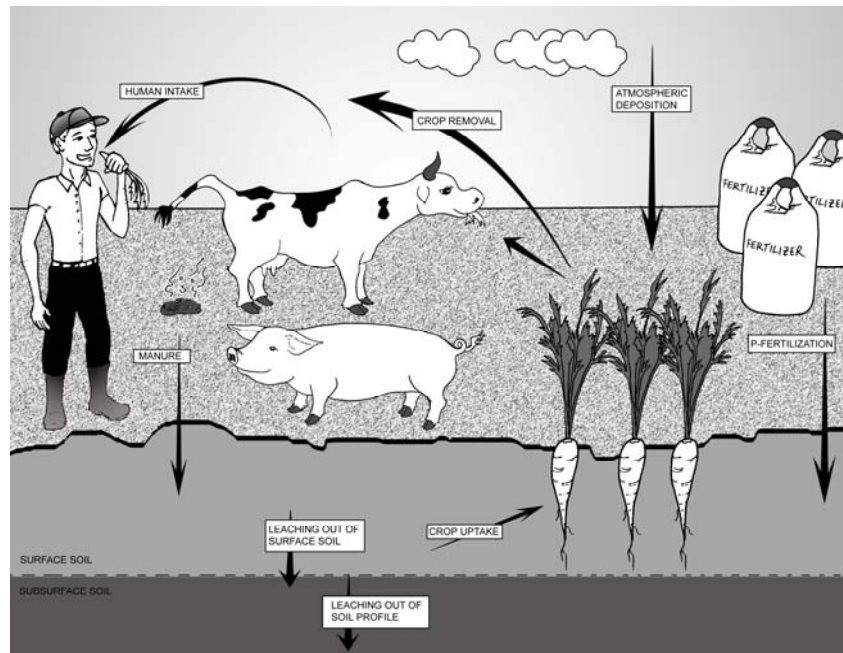


Figure 1. Cd fluxes in Swedish arable land. Illustration: Erik Kohlström

The additional Cd originates from atmospheric deposition, phosphorous (P) fertilizers, limestone and imported feed (figure 1.) (McLaughlin and Singh, 1999; Eriksson, 2009). The rate of increase seems to decline due to the fact that less Cd is circulating in society. A large amount of the Cd coming by air pollution originates from mining- and metal industry which deals with zinc (Zn) (Eriksson et al., 2005). Nowadays industries have better cleaning systems for their emissions (Eriksson et al., 2005) and the Cd that some years ago was used in batteries and paint has been replaced by other less harmful substances (Olsson et al., 2005).

As for the P-fertilizers, at least in the Western world, they contain less Cd than before (Jansson, 2002). In Sweden there is, since the 1<sup>st</sup> of January 1994, a tax on P fertilizers containing more than 5 mg Cd/kg P and also restrictions concerning the import of fertilizers that contain more than 100 mg Cd/kg P (since 1 of January 1993) (Anonymous, 2003). This has decreased the amount of Cd added to soils by fertilizers.

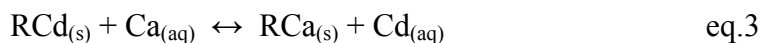
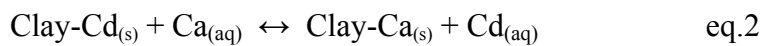
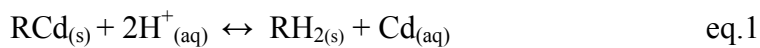


The Swedish government has declared environmental objectives, which aim to decrease the human impact on nature. One of these objectives concerns the utilisation and transfer of heavy metals to arable land (Länsstyrelsen i Skåne, 2009). This will probably contribute to an even more restricted utilisation of Cd.

### ***Cadmium availability in soils***

Cadmium can occur in three different forms in the soil. As solid precipitates, associated to soil components and dissolved in the soil solution. The most common form is associated to soil components, only 1% is found in the soil solution (Jansson, 2002). The solubility is affected by many soil factors there among pH. Low soil pH often results in high amounts of soluble Cd (Eriksson et al., 2005). Other soil factors of importance are clay- and organic matter content, amounts of Al-, Fe-, and Mn-oxides/hydroxides and cationic exchange capacity (Jansson, 2002). Since Cd is relatively weakly bound in the soil, compared to other heavy metals, about 10 to 40% can be considered exchangeable (Eriksson, 2009).

The dissolved Cd may be present as free, hydrated cations or in complexes (Traina, 1999). The complexes can be formed with organic (eq.1 and eq. 3) and inorganic matter (Jansson, 2002). It may be electrostatically bound to oxides- and clay particles (eq. 2). Therefore, the content of organic matter and clay is of great importance for the amount of free Cd<sup>2+</sup>. In equation 2 and 3, Ca<sup>2+</sup> and Zn<sup>2+</sup> can function as competitors to Cd<sup>2+</sup> and exchange Cd from the binding sites. An addition of Ca ions, for example by liming, may therefore force more Cd<sup>2+</sup> into the soil solution and thereby make it plant available.



## ***Uptake by plants***

The mechanisms behind Cd uptake in plant roots are not fully known, but the theory is that it is taken up in the same manner as Ca. However, the exact mechanism of Ca uptake is not known either. Two transport ways are suggested, symplastic and apoplastic transport (White and Broadley, 2003). It is likely that the symplastic, which is selective, is involved in Ca fluxes for cell signalling and the non-selective apoplastic is for transporting Ca in larger quantities to the shoot.

The apoplastic uptake seems to take place just behind the root tip and the regions where the lateral roots are initiated. At these points the Casparian strip is absent or disrupted, and ions such as  $\text{Ca}^{+2}$  can pass. This uptake is relatively non-selective between divalent cations which mean that toxic substances such as Cd also may pass into the plant (White and Broadley, 2003). In a study on wheat it was observed that  $\text{Zn}^{+2}$  entered root cell plasma membrane vesicles through  $\text{Ca}^{+2}$  channels (Welch and Norvell, 1999). Due to the chemical similarities between  $\text{Zn}^{+2}$  and  $\text{Cd}^{+2}$  it is likely that  $\text{Cd}^{+2}$  could enter through this pathway.

Cd uptake varies between plant species as well as between different cultivars within the same species (Jansson 1994; Olsson 1998). Whether the highest concentrations of Cd in a plant are found in the root or the shoot seems to be species dependent. Zheng et al. (2008) made a comparison between radish and carrots and found that carrots accumulated a larger part of the absorbed Cd in the taproot, compared to radish. This was thought to be due to the thinner xylems in carrots.

There seems to be differences in concentrations within the root of a carrot. According to analysis made by Olsson (1998), the parts just under the peel have higher concentrations than what is found 7-10 mm in. In the marrow the concentration again increases. Peeling the carrots reduces the Cd concentrations with 5-10 percents.

## ***How to prevent/decrease Cadmium uptake***

There are many factors influencing Cd uptake, for example plant species and cultivars (Zheng et al., 2008; Alexander et al., 2006; Stolt et al., 2006; Michalska and Asp, 2001), anthropogenic input, agricultural praxis, and precipitation (Eriksson, 2009). As mentioned earlier also soil properties, such as texture, pH, Cd, and Zn concentrations, organic matter and complexing ligands affect the uptake due to their influence on Cd availability.

The highest Cd concentrations in crops tend to be found in soils with low pH, low organic matter, high Cd and low or high Zn levels (Jansson, 2002). Moreover the plant itself affects the uptake of Cd. Different root activities affect the rhizosphere and thereby the Cd uptake. Activities that may affect the uptake is root efflux of  $H^+$  (eq. 2), phenolic compounds, organic acids and nonprotein amino acids. Since there are many factors affecting the Cd concentrations in the plants, the concentrations within a crop will differ between years and growing sites.

To decrease the amount of Cd transferred to our food there are some aspects to take into account. If possible, cultivate on soils with low Cd levels and choose cultivars that are less prone to take up Cd. The ability to take up Cd can be an aspect to consider during breeding of new cultivars. Where crops are grown at fields with low Zn status an addition of Zn fertilizers can decrease the uptake of Cd (Oliver et al., 1994) since  $Zn^{2+}$  is a competitor to  $Cd^{2+}$  for binding sites.

Liming has been suggested as a method to decrease the uptake of cadmium, but the results are inconsistent. Some studies show that Cd uptake decreases (Page et al., 1981; Jansson, 2002) with increasing pH while others showed an increase (Smith, 1994; Singh et al., 1995; Jansson, 2002) or no effects at all (Smith 1994; Singh et al., 1995; Sparrow and Salardini, 1997; Jansson, 2002).

Liming will not only result in changed soil pH, other factors that might be of importance are altered as well. Adding lime, for example  $CaCO_3$ , will result in rising pH since  $H^+$  is consumed. The result of the reaction will be free Ca ions; hence the amount of  $Ca^{2+}$  will increase in a limed soil. Since  $Ca^{2+}$  and  $Cd^{2+}$  ions are similar and thought to be taken up by

plants in the same way, an increase in Ca might have an effect on the Cd uptake due to competition. Liming also result in a change in ion strength, which might affect the Cd uptake.

## ***Aim***

The aim of this Master thesis is to study the effects of liming and Ca ions on Cd uptake and concentration in carrots. The ambition is to find out more about liming as a possible method to regulate the Cd uptake and if possible decide whether changes in pH, Ca concentration or ion strength are the main factor controlling Cd uptake.

## **Materials and methods**

Two experiments were conducted. A long-term experiment where the carrots were grown in a peat/sand substrate with added Cd, and one short-term experiment where they where pre-grown in vermiculite and nutrient solution. After three weeks their uptake of Cd was tested in a nutrient solution containing Cd labelled with the radioactive isotope Cd<sup>109</sup>.

The results were statistically analysed using analysis of variance with Tukey's method for multiple comparisons (general linear model in Minitab). Due to the mistake of not adding any Cd in one of the pots in the high Cd treatment at pH 6.6, this replicate was excluded in all calculations.

## ***Plant material and growth conditions***

The plant material used was *Daucus carota* L ssp. Sativus cv. Nestor "summer carrot" (Weibulls). The experiments were conducted, and the plants were grown, in a climate chamber where they received 400  $\mu\text{mol m}^{-2} \text{s}^{-1}$  during 16 hours per day. The temperature was 20 °C and 18 °C for day and night respectively. The relative humidity was constant at 70%. The nutrient solution used in both the pot experiment and the uptake experiments was Sonneveld lettuce nutrient solution (SNS). It was composed as follows, macronutrients: (mM) 10 KNO<sub>3</sub>, 4.5 Ca(NO<sub>3</sub>)<sub>2</sub> x 4H<sub>2</sub>O, 1 MgSO<sub>4</sub> x 7H<sub>2</sub>O, 1 KH<sub>2</sub>PO<sub>4</sub>, 1 Na<sub>2</sub>HPO<sub>4</sub>, 1.25 NH<sub>4</sub>Cl and micronutrients; ( $\mu\text{M}$ ) 40 Fe-EDTA, 5 MnSO<sub>4</sub> x H<sub>2</sub>O, 4 ZnSO<sub>4</sub> x 7H<sub>2</sub>O, 30 H<sub>3</sub>BO<sub>3</sub>, 0.75 CuCl<sub>2</sub> x 2H<sub>2</sub>O, 0.5 Na<sub>2</sub>MoO<sub>4</sub>. The desired pH values for the solutions were set using NaOH and/or H<sub>2</sub>SO<sub>4</sub>.

## Long-term substrate experiment

The substrate was a blend of 1/3 volume units sand (Baskarpssand B55, 0.54-0.57) and 2/3 peat (Hasselfors garden, Solmull, unfertilized) which were mixed in a cement mixer. The substrate was chosen because of its low pH. It is difficult to lower soil pH with a long term effect. Therefore, to be able to test the Cd uptake in low pH, a substrate with naturally low pH, such as peat, was required.

The experiment was conducted with two concentrations of Cd, 0.23 mg Cd/kg soil and 2.3 mg Cd/kg soil. Henceforth, they will be referred to as low and high Cd treatment, respectively. The low Cd treatment was chosen in order to correspond to the mean Cd level of Swedish arable land. To see if the effect of liming was different in a high Cd soil, a treatment with ten times higher Cd was also conducted. For each Cd concentration, three different treatments were carried out with five replicates each. One treatment was not limed and two were given limestone containing 20% Ca. To reach the desired pH levels 4.6, 5.6, and 6.6 they were given 0, 4.5, and 18 g limestone per pot respectively (table 1). All pots were given 500 ml SNS with double concentration, pH set to 4.6 for the unlimed treatment and 5.6 for the limed ones. The amount of SNS was based on the N-recommendations for field grown carrots with a calculated yield of 100 tonnes per hectare (YARA 2009). The nutrient solution was added as double concentration SNS to avoid possible nutrient leakage if the pots was water saturated.

Substrate, SNS and lime, if any, were mixed in a bucket and filled in rose pots with a volume of 3 L. At the top, 2 cm sand was placed and ten seeds were sown in a circle. The pots were randomly placed in the climate chamber. After germination, three plants were kept. They were selected according to even size as well as position in the pot. Irrigation was applied twice a week to 60% of pot capacity. On the second irrigation occasion in week six, and at the following irrigation occasions, they were given 100 ml of SNS full strength due to visible nutrient deficiency.

Table 1. Experimental setup for long-term soil experiment. In the table added limestone (g/pot), target pH, and the measured pH after harvest is shown.

	Low Cd			High Cd		
	0	4.5	18	0	4.5	18
Limestone, g/pot						
Target pH	4.6	5.6	6.6	4.6	5.6	6.6
Measured pH	4.2	4.7	7.0	4.4	4.9	7.0

After eleven weeks the carrots were harvested, and fresh weight of haulm and root were noted. To remove soil, the roots were rinsed under water and lightly scrubbed, before they were cut in to pieces. One cm of the top was cut off to prevent soil contamination. The plant material was placed in a drying cabinet at 70°C for 48 hours. Roots were ground and the haulms were crumbled to achieve a pooled sample.

About 0.5 g of the samples was wet combusted in 10 ml of 65% nitric acid (HNO<sub>3</sub>) in a microwave oven under high pressure and temperature. The content of macronutrients was analysed by inductively coupled plasma atomic emission spectrometry (ICP-AES) and the content of Cd was analysed by inductively coupled plasma mass spectroscopy (ICP-MS).

### **Soil analyses**

To see if there were any differences in Cd availability between the pH levels a DTPA extraction was conducted after harvest. DTPA was used since it is a rather weak extraction method and might give an indication regarding the Cd availability. The solution is a mixture of 14.919 Tetraethylammounim TEA, 1.9670 g diethylenetriaminepentaacetic acid (DTPA) and 1.4700 g calcium chloride (CaCl<sub>2</sub> x 2H<sub>2</sub>O). The soil in each pot was mixed to get a pooled sample, sieved (2.5 mm) and air dried. From this 10 g soil was taken, mixed with 20 ml DTPA solution and put in a shaker for two hours. The supernatant was decanted and filtered through filter paper (Whatman 41, 20–25 µm). The liquid was again filtered (0.45 µm) and analysed by inductively coupled plasma mass spectroscopy (ICP-MS).

For pH measurements soil was taken in the middle of the pot after harvest. It was sieved (2.5 mm) and air dried. From this 30 ml was taken and shaken with 150 ml distilled water (1:5) for one hour. The pH was then measured immediately in the supernatant.

### **Short-term nutrient solution experiment**

To be able to study how the parameters Ca, pH and ion strength affect the Cd uptake separately from each other, two experiments in nutrient solution were conducted. Two pH levels were tested, 5.6 and 6.6, and for each pH three levels of calcium nitrate (Ca(NO<sub>3</sub>)<sub>2</sub>) were tested, 2.25, 4.5, and 9.00 mM. Each treatment had five replicates. The difference in Ca(NO<sub>3</sub>)<sub>2</sub> levels gave rise to variations in ion strength. In one experiment the differences in ion strength were compensated with potassium nitrate (KNO<sub>3</sub>) (table 2). Other Ca and

potassium (K) salts were also discussed, for example chloride (Cl) and phosphate (P). Nitrate (N) and potassium were chosen since they were thought to have the least impact on the Cd uptake.

Seeds were germinated and grown in vermiculite. After two weeks, 72 of them were rinsed from vermiculite in distilled water and transferred to two plastic boxes, each containing 25 L of half strength SNS. While growing in the box they were continuously aerated. After one week the solution was changed to full strength SNS. They were also given new SNS the day before conducting the experiment.

In the uptake experiments SNS with some modifications was used. It contained 0.05  $\mu\text{M}$  Cd labelled with 740 kBq Cd<sup>109</sup>. To avoid complex formation with Cd, Fe-EDTA was excluded from the solutions.

Table 2). Concentrations of Ca(NO<sub>3</sub>)<sub>2</sub> and KNO<sub>3</sub> (mM) in the treatment solutions used in the short-term nutrient solution experiment. K<sup>+</sup>, Ca<sup>2+</sup>, NO<sub>3</sub><sup>-</sup>, and NH<sub>4</sub><sup>+</sup> concentrations are also shown. All the solutions were tested at two different pH, 5.6 and 6.6.

<i>Ion strength compensated, mM</i>					
Ca(NO <sub>3</sub> ) <sub>2</sub> ,	KNO <sub>3</sub> ,	K <sup>+</sup> ,	Ca <sup>2+</sup> ,	NO <sub>3</sub> <sup>-</sup>	NH <sub>4</sub> <sup>+</sup>
2,25	23,5	23,5	2,25	28	1,25
4,5	19	19	4,5	28	1,25
9	10	10	9	28	1,25
<i>Ion strength not compensated, mM</i>					
2,25	10	10	2,25	14,5	1,25
4,5	10	10	4,5	19	1,25
9	10	10	9	28	1,25

The experiment was conducted when the plants were three weeks old. The roots of each randomly chosen plant was placed in a beaker containing 250 ml solution (SNS +Cd) for three hours, followed by 20 minutes in a washing solution. The washing solution was SNS with ten times higher Ca(NO<sub>3</sub>)<sub>2</sub>, 45 mM.

When washed, the roots and shoot were separated, blotted with a napkin and placed in a plastic cup. After air drying for 24 hours they were transferred to a drying cabinet where they were kept at 70°C for 48 hours. The dry weight was noted and the material was wet combusted in 10 ml of 65% HNO<sub>3</sub> in a microwave oven under high pressure and temperature.

The sample was diluted with distilled water to 50 ml and transferred to a test tube. From the test tubes 2.5 ml of sample solution was transferred to a tube and mixed with 2 drops of hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) and 5 ml scintillation liquid (Quicksafe A). These were run in a liquid scintillator to analyse the radioactivity of the sample.



## Results

### *Long-term soil experiment*

#### **Plant growth**

The carrots germinated well in all treatments. After about six weeks both young and older leaves in random pots from the limed treatments showed symptoms of nutrient deficiency. Therefore they were given 100 ml SNS together with the irrigation water. The roots developed well in all treatments except the ones not receiving any lime.

At harvest the fine roots of plants grown at target pH 5.6 and 6.6 were visible through the hole at the bottom of the pots. For target pH 4.6 this was not the case, the fine root and the taproot had developed only in the sand on top of the substrate mix. However, the haulm of these plants had a normal appearance. At harvest the fresh weight of haulm and roots were noted and the roots grown in unlimed treatments were clearly smaller than roots from the other treatments.



Figure 2. Photo of a carrot grown at pH 4.6 in low Cd soil. *Foto: Anna Holmkvist*

There was no big difference in haulm fresh weights between the liming treatments grown in low Cd soil. The smallest were found in target pH 4.6 and the largest in 6.6 (figure 3). Looking at the roots, a large difference in fresh weights was seen between target pH 4.6 and the other two, 5.6 and 6.6. The fresh weight increased three times when the measured pH rose from 4.2 to 4.7. The largest roots in high Cd soil were found at pH 6.6 with a fresh weight of 167.6 g. The three carrots in each pot, making up a pooled sample, were of even size in both low and high Cd treatment

For the plants grown in high Cd soil the result was slightly different. Just as in the low Cd treatment the root from carrots grown in target pH 4.6 had the lowest weights, but here the largest roots were grown at pH 5.6 followed by pH 6.6 (figure 4). The increase in root weight when the pH was raised from 4.4 to 4.9 was 122.5 g. As for the fresh weight of haulm, there was a small increase seen with increased pH.

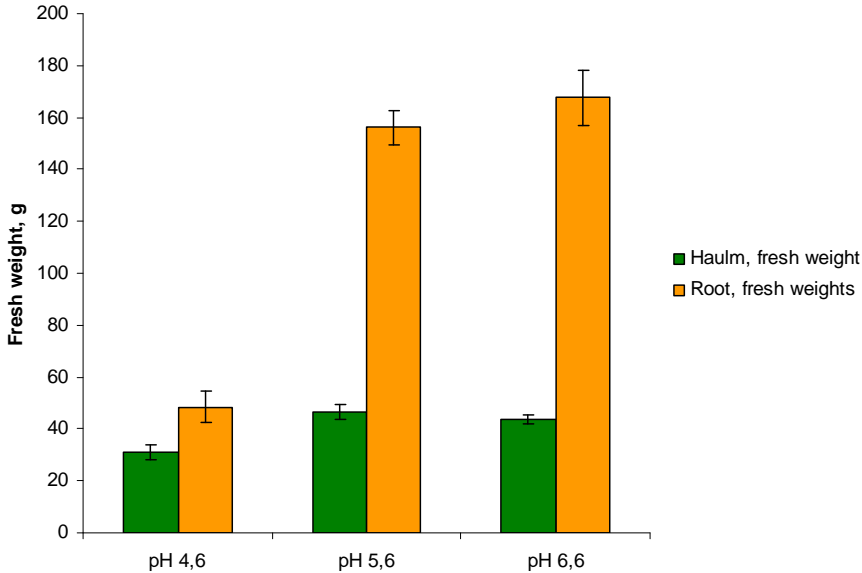


Figure 3. Fresh weight of haulm and root of carrots grown for eleven weeks in a *low* Cd soil at three different pH. (n=5 ± SE).

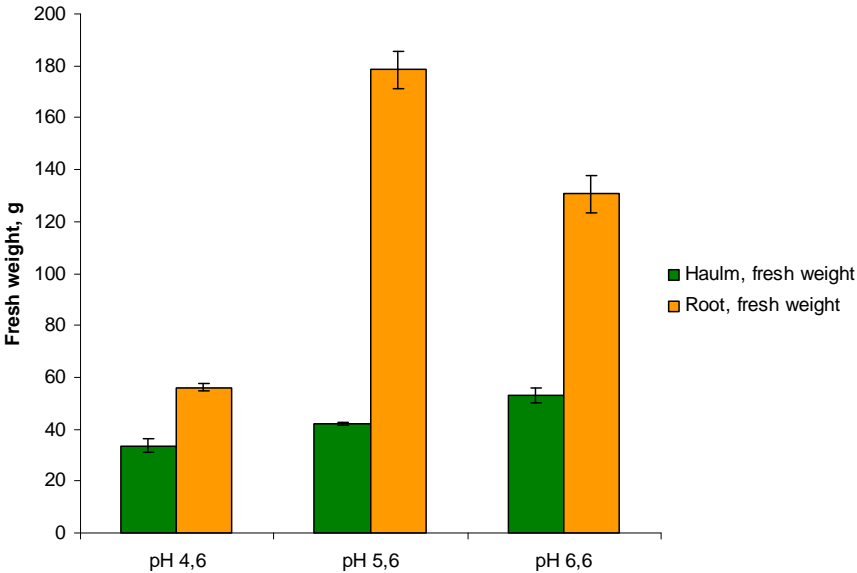


Figure 4. Fresh weight of haulm and root of carrots grown for eleven weeks in *high* Cd soil at three different pH. (n=5 ± SE, for pH 6.6 n=4).

The target pHs were not corresponding to the actual pHs measured after harvest. The calculated increase in pH, when adding the limestone, was 1 pH unit. But the result was an increase of 0.5 units between target pH 4.6 and 5.6 for both low and high Cd treatments. Between target pH 5.6 and 6.6 the increase were lager, 2.3 and 2.1 units for low and high Cd treatment respectively (table 1).

### **Cadmium concentrations in plants**

Comparing the Cd concentration of all treatments (high and low, and the three pH) and replicates, as well as haulm and root, shows a significant difference between target pH 4.6 and the others, 5.6 and 6.6 ( $p < 0.5$ ). It was also significantly shown that there was a higher Cd concentration in haulms than in roots ( $p < 0.001$ ).

Higher concentrations were found in haulms, but when considering the weight of the plant parts, one finds that a larger proportion of Cd is stored in the root due to its higher weight. Comparing the three different liming treatments in the low Cd experiment, there was a significant difference in Cd concentration between the haulms of the plants (figure 5). As for the roots there was a significantly different difference between pH 6.6 and the others.

In high Cd treatment, there were significant differences in Cd concentrations in haulms between pH 4.6 and 5.6 (figure 6). The concentration was decreasing in haulm and root at pH 6.6 compared to 5.6, but the decrease was not significant. The lowest concentration in roots was at pH 6.6, but very close to pH 4.5. For the roots the highest concentrations were found at pH 5.6 but it was not significant from the others.

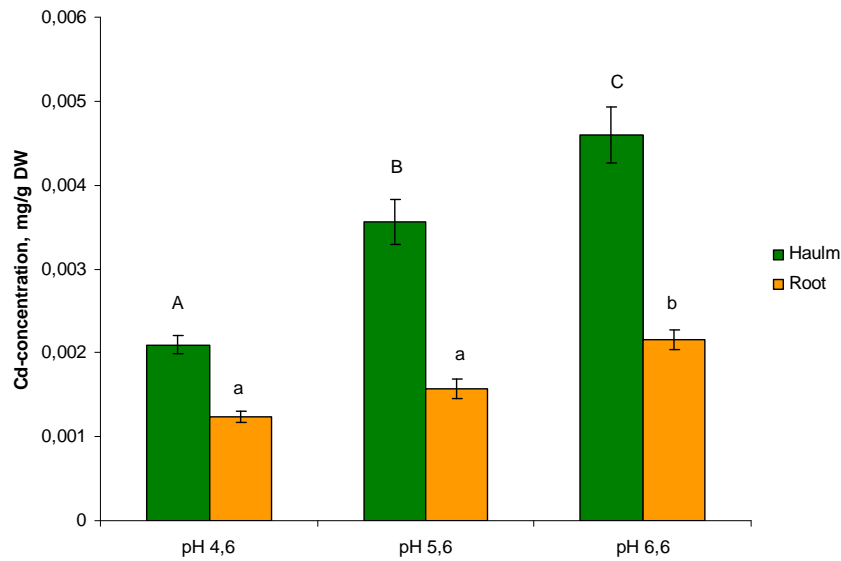


Figure 5. Concentration of Cd in haulms and roots from carrots grown for eleven weeks in a *low* Cd soil at three different pH. (n=5 ± SE).

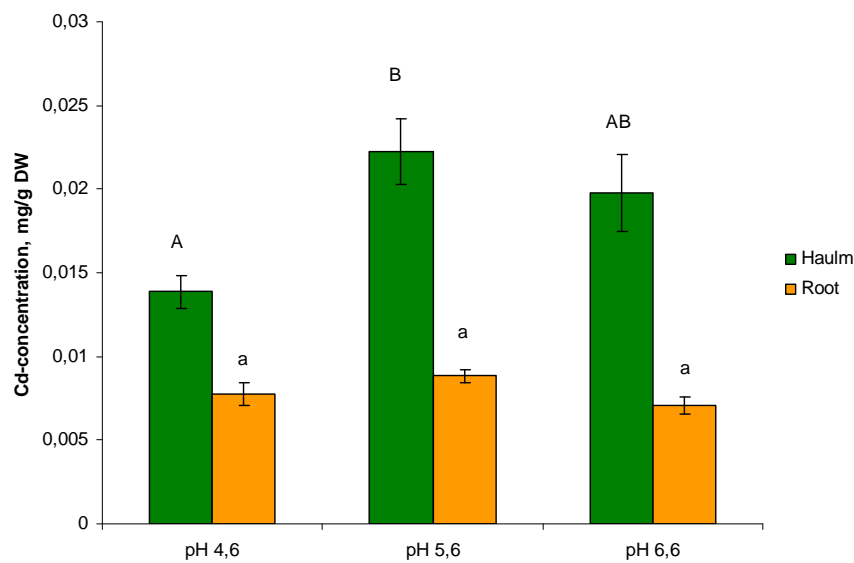


Figure 6. Concentration of Cd in haulms and roots from carrots grown for eleven weeks in a *high* Cd soil at three different pH. (n=5 ± SE, for pH 6.6 n=4).

### Calcium concentrations in plants

There were different amounts of Ca in the treatments due to different amounts of limestone added. Since the SNS contained Ca, all the treatments should have had adequate amounts of Ca, but since the plants showed symptoms of nutrient deficiency that was maybe not that case. In the haulm of the carrots grown in low Cd soil there was a significant difference seen in Ca

concentration between pH 4.6 and the higher pH values (figure 7). In the root there were significant difference between pH 6.6 and the others.

The result for Ca concentrations in haulm from carrots grown in high Cd was the same as for low Cd, a significant difference between 4.6 and the others (figure 8). For the roots there was significant difference between 4.6 and 6.6, but not between the others.

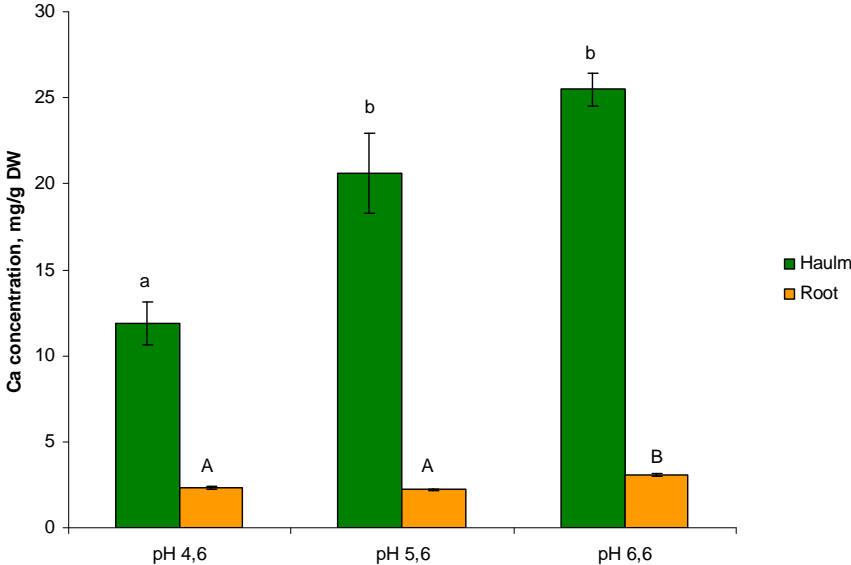


Figure 7. Concentration of Ca in haulms and roots from carrots grown for eleven weeks in *low* a Cd soil at three different pH. (n=5 ± SE).

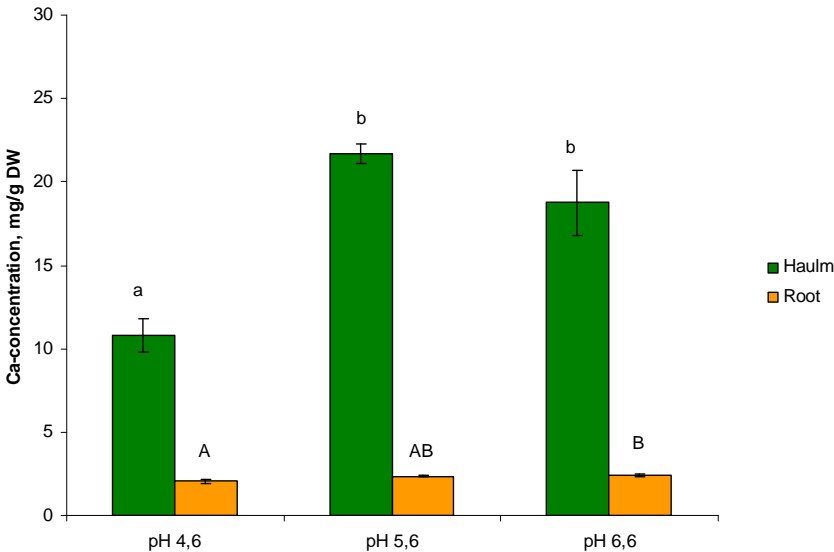


Figure 8. Concentration of Ca in haulms and roots from carrots grown for eleven weeks in *high* a Cd soil at three different pH. (n=5 ± SE, for pH 6.6 n=4).

## DTPA extraction

DTPA extraction is a weak extraction method that might give an indication of how much Cd that was plant available. In the low Cd treatment, the total uptake by plants increased with increasing pH (table 3). As for the high Cd treatment the uptake increased from pH 4.6 to 5.6 but decreased from pH 5.6 to 6.6. The amount of Cd extracted with DTPA from both low and high Cd soils decreased with increasing pH. Adding up the Cd removed by plants and the amount extracted by DTPA indicates that more Cd were available in low pH than in high. Hence when the removed Cd by plants and DTPA are subtracted from the added amount Cd in each pot, more Cd remains in the soil at high pH levels.

Table 3. Amount of added Cd in pots of low and high Cd treatment is shown. For each pH the total Cd removed by plant and DTPA extraction as well as the difference between added and removed Cd is listed.

Treatment pH	Low Cd			High Cd		
	4.6	5.6	6.6	4.6	5.6	6.6
Total Cd added/pot, mg	0,46	0,46	0,46	4,6	4,6	4,6
Uptake by plants, mg	0,0125	0,0412	0,0550	0,089	0,251	0,197
DTPA extracted Cd, mg	0,3504	0,2512	0,178	3,952	3,448	2,73
Total Cd removed, mg	0,3629	0,2924	0,2330	4,041	3,699	2,927
Difference, mg	0,0971	0,1676	0,2270	0,559	0,901	1,673

## Short-term nutrient solution experiment

In the experiment where the difference in ion strength was compensated with KNO<sub>3</sub>, there was a significant difference between 2.25 mM Ca at pH 5.6 and all other treatments except 4.5 mM Ca at pH 5.6 ( $p < 0.01$ ) (figure 11). As for the other treatments no significant differences were found, although the trend was that higher Ca concentration gave lower Cd influx at pH 5.6. At pH 6.6 the Cd influx was about the same for all Ca concentrations.

In the experiment where the ion strengths were not compensated the influx pattern was different, compared to the compensated treatment. There was no significant interaction between pH and Ca meaning that Ca concentration and pH affects Cd influx independent of each other (figure 12).

Plant size varied between the two experiments, compensated and non compensated, even though they were grown in the same way and were of the same age. Plants in the compensated experiment had an average root weight of 0.0215 g (+/- 0.0011) whereas in the non compensated the average was 0.0101 g (+/-0.0004).

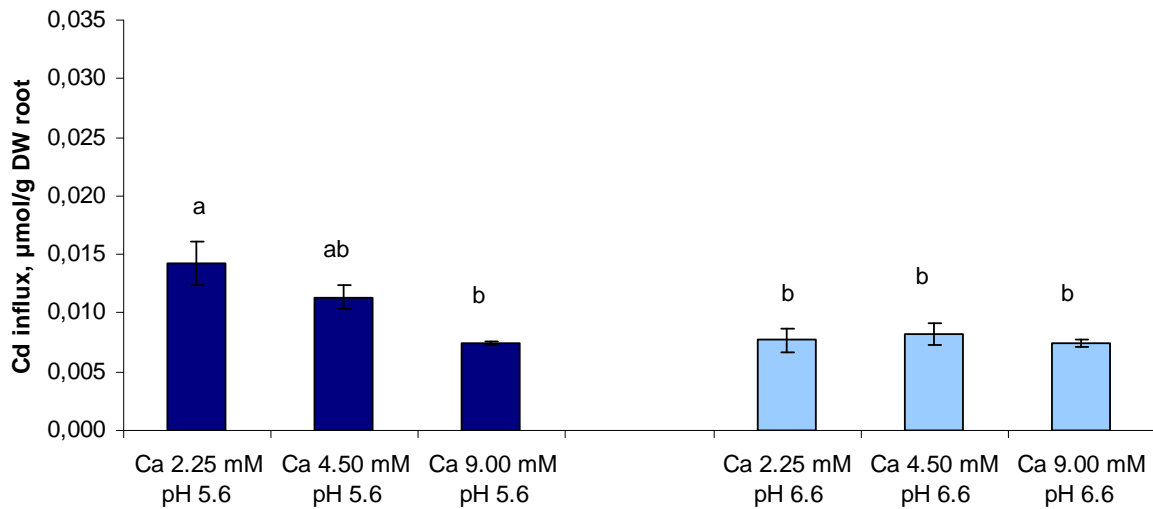


Figure 11. Cd influx in plants treated with two different pH. For each pH three different concentrations of Ca were tested. The differences in ion strength were *compensated* with  $\text{KNO}_3$ . ( $n=5 \pm \text{SE}$ )

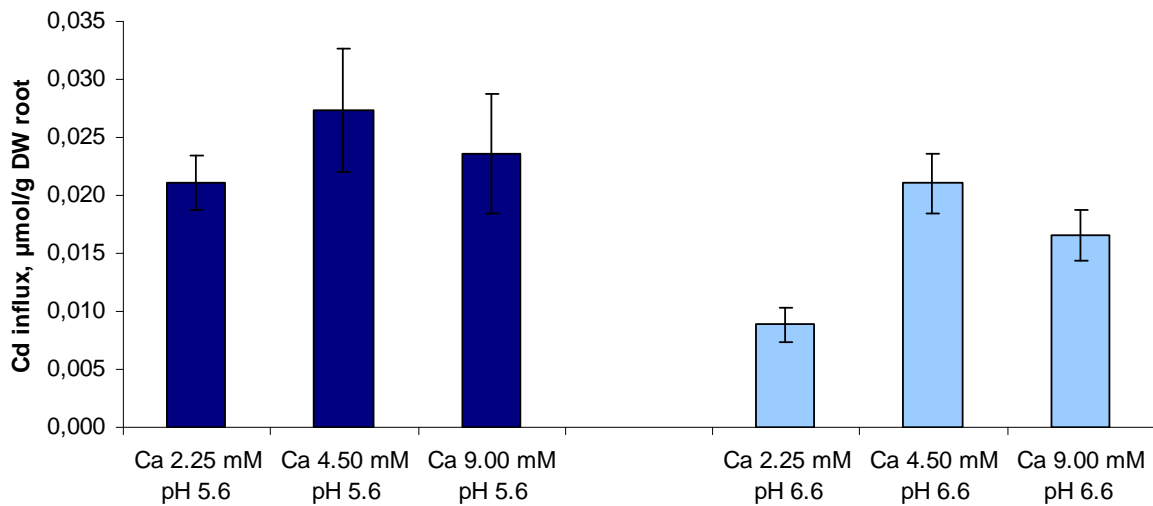


Figure 12. Cd influx in plants treated with two different pH. For each pH three different concentrations of Ca were tested. The differences in ion strength were *not compensated*. ( $n=5 \pm \text{SE}$ )

## Discussion

### *Long-term soil experiment*

#### **Plant growth**

A few weeks after germination, visible symptoms of nutrient deficiency appeared. They first became visible in the limed treatments, for both high and low soil Cd levels. Soon the symptoms were seen in all pots. The symptoms were thought to be due to deficiencies of Ca and magnesium (Mg), since the symptoms correspond well to the pictures of these deficiencies seen in Scaife and Turner (1983). However, the nutrient solution added should have supplied the plants with enough Ca and Mg during the growth. As for the limed treatments, they received additional Ca and Mg from the added lime which makes the shortage of these ions even more doubtful. The symptoms might rather be a consequence of the high concentrations of K in the SNS. The high K concentrations might have restricted the uptake of other ions due to competition.

Also the content of Cd might have contributed to the symptoms of deficiency. The findings of Shamis et al. (2007) and Cieccko et al. (2001) support this. Shamis et al. (2007) found that a concentration of 0.1  $\mu\text{M}$  Cd in nutrient solution inhibit the uptake of Ca and Mg in soybeans. Cieccko et al. (2001) found in a field study that Cd had negative effects on the uptake of Mg in spring triticale.

The low weight of the roots at pH 4.6, both in high and low Cd treatment, was probably due to the low pH. The pH measured after harvest was 4.2 and 4.4 for the low respectively high Cd treatments (table 1). In these treatments neither the taproot, nor the fine roots had penetrated the soil to any high extent. Instead they were growing in the sand layer on the surface where the pH probably was higher. The high content of  $\text{H}^+$  at low pH may be toxic to plants and affect the architecture of the roots. For non-tolerant species this will lead to restricted growth (Kidd and Proctor, 2000). In the long-term experiment already a small increase in pH, from 4.2 to 4.7 in the low Cd and from 4.4 to 4.9 in the high Cd treatment, gave a significant increase in root fresh weight (figure 3 and 4). For carrots grown in low Cd treatment the highest weights were found at pH 7.0. This was not the case for carrots grown in the high Cd treatment, where the largest roots were found at pH 4.9. This was a bit



strange, since carrots normally prefer a soil pH of 6.5-7.0 (Anonymous, 1995). A possible explanation might be that moderate levels of a toxic substance sometimes stimulates plant growth (Asp, 2009 pers com.) but at a certain level the growth will be restricted (Asp, 2009 pers com; Shamis, 2007).

### **Cd uptake and availability**

For all treatments in the soil experiment, haulms had higher concentrations of Cd than taproots (figure 5 and 6). This corresponds to the findings of De Pieri et al. (1996), who analysed the Cd content of some economically important crops grown in three different regions in Lower Fraser Valley of British Columbia. Carrots almost always had higher Cd concentrations in haulm than in the taproots. This was also the case for Cd concentrations in leaves and roots of cauliflower, cabbage, turnip and corn.

Trials conducted by Jansson (2002) showed that Cd solubility was decreasing with increasing pH, which was also the case for the soil experiment conducted in this thesis. The total amount of available Cd was significantly higher at low pH than at high (table 3). This might be due to the fact that soils with more free  $H^+$  in the soil solution may exchange Cd from its binding sites so it becomes more plant available (eq. 1). However, the highest Cd availability in soil coincided with the lowest Cd concentrations in plants. For the low pH treatment this might be explained by the poor growth of plants. Since the roots did not penetrate the soil, nutrients and Cd were less available to the plant. In the low Cd treatment carrots grown at pH 5.6 had lower Cd concentrations than plants at pH 6.6 even though the availability was higher at pH 5.6 (table 3 and figure 5). These results can not be explained with poor growth since the plants developed well in these treatments. In high Cd treatment the Cd availability, as well as the Cd concentration in plants, decreased from pH 5.6 to 6.6 (table 3 and figure 6).

The competition between Cd and other ions, such as Ca, might have affected the uptake. However, since no decrease in Cd concentrations could be seen with increasing Ca concentrations this might not be the case in my experiments. The plant concentrations of Cd and Ca follow each other in all treatments and are well correlated to the plant weights (figure 4,5,6,7, and 8). When the fresh weights of roots increased, so did the concentrations of Ca and Cd. This indicated that plant growth might have had a larger impact on the Cd uptake than pH and Ca concentrations.

The fact that the plants suffered from nutrient deficiency due to high K concentrations might have had an affect on the uptake of Ca and Cd. Deficiency of micronutrients may trigger the release of exudates, such as  $H^+$  and organic acids (Jansson, 2002), from roots in order to increase the nutrient availability. This will not only make the micro nutrients, but also the Cd, more available for uptake.

### ***Short-term nutrient solution experiment***

In both the ion strength compensated and the non compensated treatment the Cd influx was significantly higher at low pH. This corresponds to the findings of Ohya et al. (2007), who tested soybean plants in nutrient solutions and found that the Cd concentrations in every plant tissue was higher at pH 4.5 than in 6.5, as well as with the results of Matt (1976) where lettuce and oat, grown in nutrient solution, had higher Cd concentrations in low pH compared to high. However, Larsson-Jönsson, (2009) obtained the opposite results when testing the Cd influx in potatoes. For the tested pH 4.5, 5.5, and 6.5, the Cd influx increased with increasing pH.

Results from the ion strength compensated experiment at pH 5.6 indicate that higher Ca concentrations gives lower Cd uptake. This might be explained by increased competition between  $Ca^{2+}$  and  $Cd^{2+}$ . For pH 6.6, in the compensated treatment, there was no significant difference between the three Ca concentrations indicating that Ca concentration is of less importance at higher pH.

The results from the non-compensated experiment were different compared to the compensated. In this experiment there was no connection between Ca concentrations and pH. Those results indicate that both concentration of Ca and pH had an impact on the Cd influx independently from each other.

That the Cd influx in compensated and non-compensated solutions containing 9 mM Ca was different was notable. Since Ca 9 mM was acting as a reference point for ion strengths, those solutions were supposed to be equal but generated significantly different Cd influxes. However, there were many factors differing between the compensated and non compensated experiment, making it difficult to say whether the variation in Cd influx was due to difference in ion strength or some other factor.

In the compensated experiment the concentration of both Ca and K differed (table 2). Potassium is not thought to have any impact on the Cd uptake but it gave rise to small differences in ion strength which might have an effect on the Cd uptake. In the non compensated experiment both the concentrations of Ca and  $\text{NO}_3^-$ , as well as the ion strength, were varying (table 2). According to Larsson-Jönsson, (2009) both N concentration and N source might affect Cd influx. The concentration of  $\text{NH}_4^+$  was constant between the solutions but since the amount of  $\text{NO}_3^-$  differed, the proportion of N coming from  $\text{NH}_4^+$  varied. This might have had an effect.

Even though the plants from the compensated and non compensated experiments were grown in the same climate chamber and were of the same age, the plant size was very different when the experiments were conducted. The plants in the compensated experiment were on average the double size of those in the non compensated. One explanation to the difference might be that the intensity of the lamps was changing due to new fluorescent lamps. The amount of light was not measured during the periods of plant growth. Another aspect to consider is that the two experiments, compensated and non compensated, were conducted at two different occasions.

### ***Concluding remarks***

The general recommendation is to lime arable land in order to decrease Cd uptake by plants. In the long term experiment the Cd uptake increased with increasing pH. However, since the concentrations of Cd and Ca follow each other in all treatments, and were well correlated to the plant weights, it seems like the total Cd uptake as well as the Cd concentration in the plants was mostly affected by plant growth. When it comes to applying the results from the long term experiment, there are too many factors differing for the results to be applicable on field conditions. Both organic matter and clay are known to affect the solubility of Cd. The fact that the substrate used contained a high amount of peat and no clay have certainly influenced the outcome of the experiment. A natural soil will probably not act in the same way as the substrate mix in the trial. There are other factors of importance in the experiment which make the comparison with a field trial impractical. When liming a field the lime is mixed into the top soil, whereas in the pot experiment the lime was evenly distributed in the soil volume. The longtime effects of limestone were not considered during the experiment since it was only running for eleven weeks.

The purpose of the nutrient solution experiments was to try to get further information about how Ca levels and ion strength affect the Cd uptake. However, so many factors were differing between the compensated and not compensated treatment making it difficult to draw any clear conclusions. But results from both experiments indicate that the Cd influx was larger at low pH than at high. It might also be that the effect of Ca concurrence is of more importance in low pH. Still, these results might not be valid for a field since soil solution is much more complex than nutrient solutions.

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