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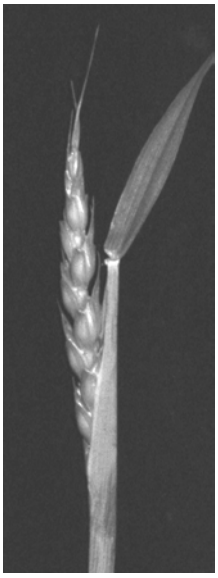
Cadmium uptake in willow (*Salix viminalis* L.) and spring wheat (*Triticum aestivum* L.) in relation to plant growth and Cd concentration in soil solution

Eva Nylund



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Emergo 2003:7
MSc thesis
ISSN 1651-7210

Abstract

Eva Nylund. 2003. Cadmium uptake in willow (*Salix viminalis* L.) and spring wheat (*Triticum aestivum* L.) in relation to plant growth and Cd concentration in soil solution. Swedish University of Agriculture Sciences. Department of Soil Sciences.

The cadmium (Cd) concentration in agricultural soils has increased during the last decades. This toxic heavy metal is allocated to edible plant parts and moves upwards in the food chain, e.g. via cereals and vegetables. Plant uptake has been investigated in many species. For wheat, most studies have focused on the concentration in the grain, not, as in this study, on total uptake.

The aim of this project was to determine the uptake of cadmium (Cd) in clone 78183 of willow (*Salix viminalis* L.) and two spring wheat (*Triticum aestivum* L.) cultivars, cv Dragon and cv Vinjett, as a function of both crop growth parameters, and Cd concentration in the growth medium. The correlation between Cd concentration in the soil and in the soil solution was also investigated. The concentrations in the growth medium were within the range of naturally occurring concentrations of Swedish agricultural land.

The experiment was carried out as a pot experiment under controlled conditions in a growth chamber. A blend of vermiculite, perlite, fine sand and fine gravel was used as growth medium, which was applied with cadmium sulphate to the concentrations of 0.00, 0.10, 0.25 and 0.40 µg Cd g^{-1} dry soil respectively. Nutrients were supplied at an addition rate of 0.15 day^{-1} (i.e. the amount increased with 15% per day) to maintain the crop growth rate at steady state. Plant biomass above and below ground was measured by three harvests. The time of the experiment was six weeks. The concentration of Cd in the plants, soil and soil solution was analysed.

Cd uptake in plants was correlated to the Cd concentration in soil and in soil solution. Both species increased their uptake with increasing soil concentrations, but there was no significant difference in total uptake between the crops. Plant biomass production also affected the Cd uptake. When plants were grown in soil with the same concentration of Cd, the amount of Cd in the plant increased as the biomass increased. However, there was a difference between species. *S. viminalis* showed to be more efficient in taking up Cd per unit of biomass compared to the spring wheat cultivars. In *S. viminalis*, the amount of Cd in the leaves was higher than that in the roots and stems. On the contrary, roots of Dragon and Vinjett had higher amounts of Cd than the shoots.

The duration of growth, as well as concentration in the soil, influenced the uptake of Cd. Plants grown in low soil concentration for a long period may take up the same amount of Cd as plants grown in a high soil concentration during a shorter period. The conclusions were that the plant uptake increased as the concentration in the soil solution increased as well as the duration in time influenced the amount of Cd taken up. These results may be used for development of simulation models to predict plant uptake and to test the ability of plants to clean contaminated soils.

Sammanfattning

Kadmiuminnehållet (Cd) i åkermark har ökat under de senaste decennierna. Denna giftiga tungmetall allokeras till ätbara växtdelar och vandrar uppåt i näringskedjan t.ex. via vegetabilier. Upptaget av Cd i växter har undersökts för flera arter. Vad gäller vete har fokus legat på metallkoncentrationen i kärnan och inte som i denna studie, det totala upptaget.

Syftet med detta projekt var att bestämma upptaget av kadmium (Cd) hos korgvide (*Salix viminalis* L.) klon 78183 och de två vårvetesorterna Dragon och Vinjett (*Triticum aestivum* L.) som en funktion av tillväxtparametrar och kadmiumkoncentration i odlingsmediet. Sambandet mellan koncentrationen i odlingsmediet och i marklösningen undersöktes också. Till skillnad från många andra laboratorieförsök var koncentrationerna i odlingsmediet var i samma storleksordning som i den svenska åkermarken.

Försöket utfördes som ett krukexperiment i klimatkammare under kontrollerade förhållanden. Odlingsmediet bestod av en blandning av vermikulit, perlit, fingrus och finsand. Kadmiumsulfat var tillsatts till koncentrationerna 0,00, 0,10, 0,25 och 0.40 µg Cd g^{-1} torrsubstans. Mineralnäring tillsattes i en relativ tillförselhastighet av 0.15 dag^{-1} (d.v.s. mängden näring ökades med 15% per dag) för att få plantornas tillväxthastighet i "steady state". Tre skördar utfördes där ovan- och underjordisk växtbiomassa bestämdes och Cd koncentrationen i plantorna, odlingsmediet och marklösningen analyserades. Den tredje skörden ägde rum efter fyra respektive fem veckor för korgvide och vete.

Resultaten visade att upptaget av kadmium i växten var korrelerat till koncentrationen i odlingssubstratet såväl som i marklösningen. Båda arterna ökade sitt upptag med ökande substratkoncentration, och det fanns inga signifikanta skillnader i totalt upptag mellan grödorna. Upptaget befanns vara beroende av biomassaproduktionen då mängden Cd i växten ökade med ökad biomassa vid odling i lika Cd-koncentrationer. *S. viminalis* visade sig vara mer effektiv i sitt upptag per viktsenhet i jämförelse med vetesorterna. De båda arterna allokerade Cd olika. *S. viminalis* hade, relativt sett, större mängd Cd i bladen än i rot och stam. Vårvetesorterna å andra sidan hade större mängd Cd i rötterna än i skotten.

Både växtperiodens längd och kadmiumhalten i marken visade sig vara viktig för kadmiumupptaget. Plantor odlade i substrat med låg Cd-koncentration under en lång period tog upp samma mängd Cd som växter odlade i hög markkoncentration under en kort tidsperiod. Slutsatserna var att växternas upptag ökade med både ökande koncentration i odlingsmediet samtidigt som växtperiodens längd hade inflytande på hur stor mängd Cd som togs upp. Resultaten kan användas för att utveckla simuleringsmodeller, t.ex. för att förutspå växters upptag och att testa växters förmåga att rena kontaminerade marker.

Preface

This project constitutes the final assignment in my studies required for the degree of Master of Science (MSc) in Agriculture with specialisation in plant/soil science. The choice of subject was mainly crop production and plant nutrition. During these courses arose the questions of why and how cadmium is taken up in plants, and the search for answers led to this project.

Several studies of Cd uptake have been performed on *Salix* and wheat separately, but the issue has not been fully answered. Models have been developed for simulation of Cd uptake in *Salix viminalis* (L.) (Palm, 1996; Blombäck, 2002). In the present study, both willow and spring wheat were grown, to discover whether there are differences between them according to uptake, allocation pattern and growth. It is hoped that the results will be of value for future investigations and to the further development of models for Cd uptake in crops.

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Introduction

Cadmium (Cd) is known to accumulate in agricultural soils and in food, and this issue has been intensely discussed since the 1980s. Cd is allocated to edible plant parts and moves upwards in the food chain, *e.g. via* vegetables and cereals. The heavy metal is toxic for both animals, including humans, and plants in large doses, due to its effect on enzyme activity. In animals, it may lead to kidney disorder and osteoporosis (brittleness of the bones). In plants, iron metabolism may be disturbed, which causes chlorosis and inhibition of electron transport in the photosynthetic process (Mengel & Kirby, 1982; Nationalencyklopedin, 1993a). In a new regulation the European Commission (2001) has set the maximum permissible concentration of Cd in cereals at 0.1 $\mu g\ g^{-1}$ wet mass. Bran, germ, wheat grain and rice may have a Cd content of 0.2 $\mu g\ g^{-1}$ wet mass.

Some soil parent material contributes to a high background content of the soil, since Cd occurs naturally in minerals of igneous and sedimentary rocks at a level of 0.08–0.5 $\mu g\ g^{-1}$ (Nationalencyklopedin, 1993a; Ross, 1994). Usually, Cd occurs together with Zn, in zincblende especially, at a concentration of 0.05–0.8%. Pure Cd minerals are rare and of no economic value. A large percentage of the agricultural soils of the world are today contaminated by Cd that originates from anthropogenic sources, such as combustion gases from industry, sludge, manure and mineral fertiliser made from raw phosphate, which often contain Cd.

In Sweden, the average concentration of Cd in the plough layer (0–20 cm) of agricultural soils is 0.23 $\mu g\ g^{-1}$ dry soil, and 80% of all Swedish agricultural soils have a concentration of 0.1–0.37 $\mu g\ g^{-1}$ dry soil (Eriksson *et al*., 1997). Most of the subsoils of Sweden have a value below 0.11 $\mu g\ g^{-1}$ dry soil. High amounts of Cd in both topsoil and subsoil were found in southern Skåne, in the county of Östergötland and in the area around Lake Storsjön in the county of Jämtland, where correlations were found between the Cd content in the soil and that in the parent material. The lowest concentrations of Cd in agricultural soil were found in the forest areas of Sweden and on the plains near Lake Vänern and the West Coast. Elsewhere in Europe, *i.e.* France and the Netherlands, the average content in agricultural soil is 0.39 $\mu g\ g^{-1}$ dry soil and 0.32–0.87 $\mu g\ g^{-1}$ dry soil, respectively (Ministry of Social Affairs, Public Health and the Environment, 1999). These values are representative for most countries in Europe. A survey of the podsol soils of Swedish forests, made by Andersson *et al.* (1991), showed that the average Cd concentration of the mor layer was 0.64 μg Cd g^{-1} dry soil. The Cd derives from atmospheric deposition and from the litter of trees that have allocated Cd to the leaves/needles.

To minimise the addition of Cd to agricultural soils, some producers of mineral fertilisers use raw phosphate low in Cd, *e.g.* Hydroagri (2003). In Sweden, farmers' organisations and cereal industries have recommended the use of fertilisers with low amounts of Cd (Cerealia, 2003; Lantbrukarnas Riksförbund, 2003; Svenskt sigill, 2003). The Swedish government prohibited in 2001 (Naturvårdsverket, 2001 SNFS 2001:5) the spreading of sewage sludge on agricultural land if the soil contained more than 0.4 μg Cd g^{-1} dry soil, since sludge is regarded as a major source of Cd to agricultural soils. In Europe, the European Economic Community (EEC) promulgated directives on limits to discharges and emissions of Cd in air, water, sewage sludge and agricultural use (Ministry of Social Affairs, Public Health and the Environment, 1999).

Aim and hypothesis

The aim of this project was to determine cadmium (Cd) uptake in one clone of willow (*Salix viminalis* L.) and two spring wheat (*Triticum aestivum* L.) cultivars, as a function of crop growth as well as of Cd concentration in the growth medium.

The following hypotheses were tested:

- That Cd uptake by the plant is proportional to Cd concentration in soil solution.
- That Cd concentration in soil solution is proportional to Cd content in the soil
- That Cd uptake is proportional to the rate of root growth
- That Cd uptake is to a greater extent related to root growth than to total plant growth.

Background

Availability of cadmium

There are various hypotheses concerning how plant uptake of cadmium is correlated to soil properties and Cd concentration in the soil solution. Experiments have shown that soil properties such as soil *p*H, clay content and organic matter (OM) influence the solubility of Cd and thereby its availability to plants (Eriksson, 1990; Eriksson *et al.*, 1996). The higher is the concentration in soil and soil solution, the higher the uptake may be. At low *p*H, Cd is exchanged from its binding sites on soil particles by hydrogen and aluminium ions and dissolved in the soil solution (*e.g.* Garcia-Miragaya & Page, 1978). The solubility declines as *p*H rises from acid to neutral, owing to the adsorption of Cd on OM and clay minerals (Eriksson, 1990; McBride, 1995). Clay particles have negatively charged surfaces that may adsorb the cation of Cd (Cd^{2+}) to a large degree (Garcia-Miragaya & Page, 1978), but the phenol and carboxylic groups of organic matter have been shown to be more effective in adsorbing Cd and in making it unavailable to plants (Eriksson, 1990). An increase in the amounts of both OM and clay content decreases the amount of soluble cadmium in soil solution, owing to the greater number of available binding sites (Eriksson, 1990; Del Castillo & Chardon 1995). Eriksson (1990) suggests that peat forms strong organic complexes with Cd, which are more important to the adsorption capacity of the soil than is the general increase in cation exchange capacity (CEC) when adding peat. Haghiri (1974) and Levi-Minzi *et al.*, (1976) considered, however, decreasing Cd uptake to be an effect of the high CEC of organic matter.

The dissolved Cd occurs mainly as Cd^{2+} but may also occur as complexes with small or large organic molecules, dispersed metal colloids such as hydroxy polymers of Fe and Al, as well as inorganic complexes such as $CdCl^+$, $CdCl_2$ and $CdSO_4$. In addition to Cd^{2+}, the latter complexes may be taken up by plants (quoted in Singh & McLaughlin, 1999).

The Cd concentration of the soil solution is correlated to the adsorbed amount of Cd on the binding sites, and this interaction can be described by a Langmuir isotherm (McBride, 1995). The Langmuir isotherm is defined as the relation between the adsorbed amount of Cd and the concentration in the solution, stated for constant soil parameters (Nationalencyklopedin, 1993b; McBride, 1995). The adsorption increases with increasing concentration in the soil solution, but will asymptotically reach saturation value. As organic matter disappears from the soil, the equilibrium concentration in soil solution increases, owing to the decrease of the adsorption maximum.

Uptake of cadmium

There are many hypotheses to explain Cd uptake. Some researchers consider that uptake is active, but most evidence points to the hypothesis of passive uptake (quated in Greger & Landberg, 1996). Active transport across cell membranes depends on metabolic energy (adenosine triphosphate, ATP) to transport ions via carriers, which are molecules that serve as binding sites (Mengel & Kirby, 1982; Marschner, 1995). Each carrier has affinity to a certain ion and regulates the content within the plant. Passive uptake, on the other hand, is independent of ATP (Larcher, 1995; Marschner, 1995). Cell walls in root epidermis and apoplast have apparent free spaces (AFS) of macro- and micropores, where passive ion uptake takes place. The macropores, called water free spaces (WFS), are freely accessible to ions as well as uncharged molecules. The micropores, called Donnan free spaces (DFS), have carboxylic groups ($R\text{-}COO^{-}$) that attract cations and serve as cation exchangers, while anions are repelled. The number of cation exchange sites, (CEC), varies among species (Marschner, 1995). Generally, the CEC per unit mass of dicotyledons is much higher than that of monocotyledons. The effective CEC of AFS decreases with decreasing external *p*H, owing to the adsorption of hydrogen ions at the binding sites.

Passive uptake of ions is affected by the valency and radius of the ion (Marschner, 1995). Uptake increases with decreasing valency of the ion; therefore the cell may more easily take up ions of low electric valency and uncharged molecules. The order of uptake is as follows:

Uncharged molecules > Cat^{+} > An^{-} > Cat^{2+} > An^{2-} > Cat^{3+} > An^{3-}

The rate of both active and passive nutrient transport through the cell membranes can be described by the Michaelis-Menten equation (Eq. 1), and depends on the two factors V_{max} and K_m (Maschner, 1995). V_{max} is the capacity factor, which denotes the maximum rate of transport when all available binding sites (carriers) are loaded. The second factor, K_m, is the Michaelis-Menten constant, equal to the ion concentration given when the transport rate is half of V_{max}. The rate of transport (v) also depends on the ion concentration of the substrate (C_s). The uptake rate is independent of the source of the element, as long as the concentration remains constant.

$$v = (V_{max}\, C_s)/(K_m + C_s) \qquad \text{Eq. 1}$$

Plants in general accumulate 0.05–2 µg Cd g^{-1} DM (Kabata-Pendias & Pendias 1992). The Cd-concentration in clones of *S. viminalis* has been estimated at 0.01–2.2 µg g^{-1} in the wood of the stem, and accumulation may be up to 20 g Cd ha^{-1} $year^{-1}$ (Greger & Landberg, 1994; Östman, 1996). From experiments, the Cd uptake of *Salix* spp. and wheat is known to increase as the Cd concentration in the soil solution increases (Eriksson, 1990; Greger & Landberg, 1996). However, the percentage of Cd taken up decreases, *i.e.* the efficiency (Cd taken up in relation to Cd supplied) of uptake declines with increased external concentration (Greger & Landberg, 1996). The toxic threshold of the soil solution has been identified as 0.5 mg Cd dm^{-3} (quoted in Dickinsson *et al.*, 1994).

Allocation of cadmium

It is hypothesised that the transport of Cd within the plant occurs in the xylem as it follows water transport upwards in the xylem (Greger & Landberg, 1996). The effects of transpiration on Cd uptake have both been confirmed (Hardiman, &

Jacoby, 1984; Pleijel, *et al.*, 1999) and not confirmed (Greger *et al.*, 2002). Andersson & Bingefors (1985) found that the uptake of Cd in wheat was lower during dry years.

Most of the Cd uptake occurs in the epidermis of the root tips (Florijn &Van Beusichem, 1993; Greger & Landberg, 1996; Koeppe, 1997). Root tips lack the Casparian band, and Cd is therefore transported apoplastically through cell walls directly to the xylem. Cations in the xylem move upwards in the negative charged cell walls of the xylem, but most (70–90%) remain in the root tissue. The reason for this is that Cd is adsorbed to negative charges on cell walls and macromolecules in cells, or is taken up by the root cell and accumulates in the cytoplasm and vacuoles. The distribution varies with plant genotypes and among *Salix* species (*e.g.* Landberg & Greger, 1994).

Research on cereals has mainly focussed on the concentration of Cd in the grain. A national survey found that the grains of Swedish winter wheat have an average Cd concentration of 0.44 $\mu g\ g^{-1}$ DW (Eriksson *et al.*, 2000). Another survey of Swedish spring wheat grain gave the result 0.56 $\mu g\ g^{-1}$ DW (Öborn *et al.*, 1995).

Clones of *Salix* spp. have been ranked according to their ability to take up and transport Cd compared to other clones and species. Clone 78183 of *S. viminalis* has been shown in experiments to have high to medium uptake capacity and low transport capacity of Cd (Landberg & Greger, 1994). For clone 78183 the highest amount of Cd was found in subsoil roots, then leaves, stem and topsoil roots in decreasing order. However, the allocation pattern varies for different clones.

Materials and Methods

Willow (*Salix viminalis* L.) clone 78183 and two spring wheat cultivars (*Triticum aestivum* L.) cv Dragon and cv Vinjett (Svalöf Weibull, Sweden) were grown at four different soil cadmium concentrations, *i.e.* 0.00, 0.10, 0.25 and 0.40 µg g^{-1} dry soil. These concentrations were within the range of Swedish agricultural soils. The growth medium consisted of 50% (volume) vermiculite (5–12 mm), 25% fine gravel (6–2 mm), 12.5% fine sand (0.6–0.2 mm) and 12.5% perlite (2–7 mm). The four fractions were separately analysed before mixing and were found to be Cd-free. The properties of the growth medium were uniform except for the 0.40 µg g^{-1} treatment, which contained larger amounts of sand and less of vermiculite and was more compact than the other.

The experiment was carried out in a growth chamber (Figure 1) (Weiss Technik, Giessen, Germany) to maintain a steady climate; 24 h illumination by 250-W lamps (Osram HQI-E, Munich, Germany), photon flux density 250 µmol m^{-2} s^{-1} (400–700 nm), air temperature 20 °C and air humidity RH 65.

The investigation was conducted as a pot experiment, using 0.8 dm^3 pots. All pots and discs used in the experiments were washed in acid water (0.1 M HCl) to avoid Cd-contamination. Each treatment had thirteen pots of each species, twelve used for harvest and one in reserve. Each pot of wheat contained three plants, while the willow pots contained only two, owing to a shortage of cuttings. All pots were placed on trolleys, which were evenly distributed in the chamber. Nutrients were added three times a week according to Ingestad & Lund (1986), at an

Figure 1. View into the growth chamber before the second harvest. The author was adding the nutrient solution to the plants.

exponential rate of 0.15 day^{-1} (the amount of nitrogen increased by 15% per day), in the proportion 100 N: 65 K: 13 P: 9 S: 8.5 Mg: 7 Ca: 0.7 Fe: 0.4 Mn: 0.2 B: 0.06 Zn: 0.03 Cu: 0.03 Cl: 0.007 Mo: 0.003 Na. Nutrients were added to provide the plants with optimum nutrient conditions for growth. The plants were irrigated five times a week with deionised water. The water was added to the discs, to avoid leaching of cadmium from the soil. Throughout the experiment, *p*H and electrical conductivity in the growth medium were checked to control the nutrient uptake.

Pre-treatments

Growth medium

Soil substrate was prepared separately for each treatment, to enable mixing to different Cd-concentrations in different treatments. Dissolved cadmium sulphate (3 $CdSO_4$ x 8 H_2O) was sprayed on the soil during mixing. To promote an even distribution of Cd in the soil substrate, it was slightly moistened before the spraying of Cd, and after spraying it was further moistened to approximately field capacity. To obtain approximately the same soil volume and porosity in all pots, *ca*. 1 dm^3 of substrate was poured into a plastic measuring jug and tapped on the table ten times until it reached a volume of 0.8 dm^3. Then it was poured into a pot and tapped again

Plant material

The *Salix viminalis* (L.) cuttings used in this experiment originated from a plantation situated at Ultuna (59°49′N, 17°40′E, 5 m a.s.l.) *ca.* 5 km south of Uppsala, Sweden. The plantation is fertilised with N, P and K, and is used only for raising material for experiments (Nordh, personal communication). The cuttings had been stored at –4 °C after harvesting. As a pre-treatment, they were laid in water for four days to saturate before they were used in the experiment. They were then cut into 5-cm long cuttings, which had a bud 0.5 cm from the top and base of the cutting. This provides good shoots and small roots, which are less sensitive to damage while being planted. The cuttings were placed for ten days in growth units, suspended in foam-rubber lids in which deionised water was circulated. During this period they developed roots and shoots. To eliminate nutrient deficiency, a small amount of nutrients (see above) was added after eight days.

The pre-treated cuttings were randomly selected and carefully planted, two by two, without damaging the roots. To ensure good contact between the roots and soil substrate, water was added. Three days after planting, nutrient addition began. At this moment, seven cuttings had stopped growing, and were replaced by new ones that also had been pre-treated as above.

To obtain uniform water content in the spring wheat kernels—important for uniform germination—the spring wheat kernels were pre-treated in deionised water for 24 hours. Six seedlings were planted in each pot and were thinned out to three plants at the start of nutrient addition. In three pots of Vinjett, no seedlings had developed, so three seedlings were then planted in each pot. They had been germinated at the same time as the rest, but were retained in the water bowl. After a few days, the new seedlings had reached the same height as the others.

Experiment

The experimental design is described in Table 1. Three harvests were made, with three randomised replicates per treatment. At harvest, one pot per replicate was used, with the exception of the first harvest, when two pots were used, owing to the small biomass. The intervals between the harvests were five to six days, since biomass theoretically doubles during this period (Ingestad & Lund, 1986). The first harvest took place on the 17th day of nutrient supply for willow, and on the 21st day for wheat. At harvest, the wheat plants were divided into root and shoot and willow into leaf, stem and root (Fig. 2a-b). Fresh masses of the crop parts and shoot length were measured for all of the replicates. Root lengths (Comair Root Length Scanner, Melbourne, Australia) of the 0.25 μg Cd g^{-1} DM treatment were measured. All material was dried at 70 °C for two days before being weighed. For some of the data analyses, the dry masses of the plant parts were added together to obtain the mass of the whole plant. After grinding (0.2 mm Retsch, Germany) the samples from the first and third harvest (Table 2) were analysed for N by dry combustion of the sample (Carlo Erba NA 1500, Italy). The analyses of the total concentration of Cd were performed with emission spectroscopy (ISO 11885, ICP-AES, Inductively Coupled Plasma Atomic Emission Spectroscopy, France) after digestion in perchloric acid and nitric acid (10:1).

Figure 2. The plants were cut into (a) stem, leaf and root for willow and (b) shoot and root for wheat. The lines indicate the cuts.

After the plants were harvested, soil samples of 50 mg were taken from the pots of the third harvest as shown in Table 2. After drying, the samples were analysed for total Cd by the same procedure as the plant analysis (see above). For sampling of the soil solution, the remaining soil substrate was saturated with deionised water for 3 hours. The saturated soil was centrifuged (J6-M1, Beckham Coulter Inc., Fullerton, USA) at 4000 rpm for 15 minutes to obtain soil solution, which was analysed for its Cd concentration without previous dilution (ISO 11885, ICP-AES).

Table 1. *Experimental design of the investigation. The treatments 0.00, 0.10, 0.25 and 0.40 mg g^{-1} dry soil had three replicas and were treated in the same way*

Species	Harvest 1		Harvest 2		Harvest 3	
	Willow	Wheat	Willow	Wheat	Willow	Wheat
Replica/treat	3	3	3	3	3	3
Pots/replica	2	2	1	1	1	1
Plants/pot	2	3	2	3	2	3

Table 2. *Analysis schedule for Cd and N concentration in plants, soil substrate and soil solution*

Treatment	Harvest 1		Harvest 2		Harvest 3	
µg Cd g^{-1} dry soil	Vegetative	Soil solution	Vegetative	Soil & soil solution	Vegetative	Soil & soil solution
0.00	-	-	-	-	N, Cd	Cd
0.10	N, Cd	Cd	-	-	N, Cd	Cd
0.25	-	-	-	-	N, Cd	Cd
0.40	N, Cd	Cd	-	-	N, Cd	Cd

Statistical analyses

The results of biomass and Cd analyses were statistically analysed using the GLM procedures in the SAS program (SAS Institute, Inc., 1987) and by calculation of linear regression using the Microsoft Excel program (Office 2000). Significance levels were $p \leq 0.05$ with least significant difference (LSD). For linear regression, an R^2-value = 0.30 was considered as relevant for significance in biological systems (Norell, personal communication). Regression lines were calculated to discover whether there was any proportionality between the amount of Cd in plant and biomass accumulation, and the Cd concentration in soil and soil solution, respectively.

Results

Experiment

The willow plants grew well and were healthy-looking. Only a few plants had dry leaves on the lower parts of the stem at the end of the experiment. Both cultivars of wheat had symptoms caused by the fungi *Drechslera tritici-repentis* and *Bipolaris sorokiniana*, originating in from seed that had not been disinfected. The symptoms were leaf spots and brown stalk bases, respectively. The fungi affected only a few plants of Vinjett, while about half of the Dragon plants had symptoms. Only a few plants of Dragon were severely damaged.

Properties of growth medium

The *p*H in the growth medium varied between 6.5 and 7.8, which indicates that the acid nutrient solution (*p*H 4) was buffered by the substrate. The initial Cd concentrations in the soil and the soil concentrations at the third harvest are shown in Table 3. The decreasing value for the 0.25 µg g^{-1} treatment indicates the plant uptake of Cd. In the 0.40 µg g^{-1} treatment, the initial Cd concentration in the growth medium was, however, lower than the value at the third harvest due to the fact that the samples were taken from the leftovers from the mixing, which may have been uneven in their Cd distribution.

The analysed Cd concentration of the soil and soil solution was highly correlated, R^2 = 0.91 (Figure 3). The concentrations in the soil solution were 0.62–4.16 ηM at the first harvest, and 0.88–6.36 ηM at the third harvest. An assumption was made that most of the dissolved Cd was present as Cd^{2+}.

Table 3. *Soil Cd concentrations (µg g^{-1} DM) in the different treatments from initial state and at the third harvest (n.a. = not analysed)*

Soil Cd Added (µg g^{-1})	Initially Measured (µg g^{-1})	Harvest 1	Harvest 2	Harvest 3
0.00	0.00 ± 0.00	n.a.	n.a.	n.a.
0.10	n.a.	n.a.	n.a.	0.074 ± 0.010
0.25	0.216 ± 0.039	n.a.	n.a.	0.186 ± 0.011
0.40	0.287 ± 0.051	n.a.	n.a.	0.327 ± 0.043

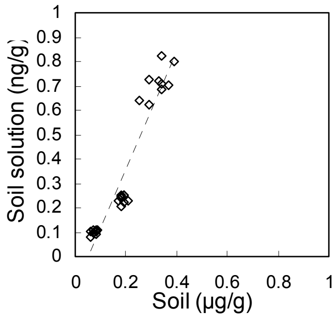

Figure 3. Cadmium concentration in the soil solution in relation to Cd concentration in soil. Results from analyses of the third harvest. ◊-----◊ = Cd concentration, y = 2.394x – 0.118, R^2= 0.91.

Growth

Results for the dry mass of the various plant parts, and the root:shoot ratio from the harvests, are shown for each species in Tables I-III in the Appendix. Root lengths were measured in the 0.25 µg g^{-1} Cd-treatment only, and are also shown for each species in Tables I-III (Appendix). The development stage of the wheat was DC 12 (two leaves) at the first harvest, DC 22 (two lateral shoots) at the second harvest and DC 43–55 (beginning of heading) at the third harvest (Zadoks *et al.*, 1974). The stem length of willow was 6–10 cm at the first harvest, 12–15 cm at the second harvest and 15–25 cm at the third harvest.

The total biomass of willow was lower than that of wheat, with the exception of the first harvest, and of Dragon at the second harvest, shown in Figure 4. Dragon had a plant biomass lower than that of Vinjett at all harvests. The roots of willow correspond to a quarter of the total plant biomass at all harvests. Dragon and Vinjett had a root biomass of 40–45% of total plant mass at the first harvest, 32–39% at the second and 23–28% at the third. The correlations between root DM and root length were high; for willow, R^2 was 0.96, for Dragon R^2 = 0.90 and Vinjett R^2 = 0.94.

The amount of total nitrogen (N) in the plants was analysed, and compared to the added amount of N, known from the nutrient schedule (Tables I-III in the Appendix). Willow took up most of the added N up to the first harvest and 70% of added N up to the third harvest. Both Dragon and Vinjett took up amounts lower than those added. The uptake of N was similar among the treatments.

The uptake of Cd had no impact on the growth of the plants, from which it is inferred that the amount of Cd in the growth medium was, as expected, not toxic. The two wheat cultivars grew at a relative growth rate (R_G) that agreed with the relative addition rate (R_A= 15%) of nutrients (Table 4), while the R_G of willow was lower than the R_A. R_G was uniform for each species, irrespective treatment, in the interval between the first and the third harvest (H1–H3). In the interval between the

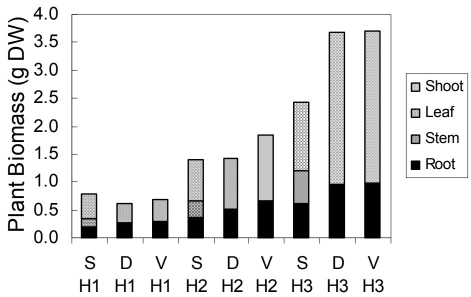

Figure 4. Plant biomass on average for the four treatments at the first (H1), second (H2) and third (H3) harvest. S = Willow, D = Dragon and V = Vinjett. Willow was divided into roots, stems and leaves while the wheat was divided into shoots and roots.

first and the second harvest (H1–H2), some of the treatments of wheat grew faster than the relative addition rate, but slowed down by the third harvest (H2–H3). Others increased their R_G and grew faster to the third harvest instead, showing the ability of plants to compensate for variations in growth rate due to different temporarily disturbances.

Table 4. *Relative growth rate (R_G) of the species at different intervals during the experiment. (H1= harvest 1, H2 = harvest 2, H3= harvest 3 and St = start of nutrition.)*

	Salix viminalis				Dragon				Vinjett			
Treatment	0.00	0.10	0.25	0.40	0.00	0.10	0.25	0.40	0.00	0.10	0.25	0.40
H1 – H2	11.8	13.9	11.7	9.0	15.2	10.8	12.2	17.1	15.0	17.2	16.2	15.3
H2 – H3	11.2	10.6	11.2	12.5	14.7	18.2	15.3	13.2	11.0	11.5	13.0	13.7
H1 – H3	10.5	11.1	10.4	9.8	14.9	14.5	13.7	15.1	13.0	14.3	14.6	14.5
St – H1	20.5	19.8	20.1	20.5	11.0	11.1	11.4	10.5	11.8	11.1	11.6	12.1
St – H3	17.1	17.0	16.9	16.9	12.5	12.4	12.2	12.2	12.3	12.3	12.7	13.0

Uptake of cadmium

Plant Cd concentrations, the amount of Cd in the plant and plant parts as well as the percentage of added amounts of Cd taken up, are shown in Tables IV-VI in Appendix. In Figure 5, the total uptake of Cd as well as the allocation of different plant parts of the third harvest, is shown for willow and Vinjett. Vinjett represents both wheat cultivars, since their uptake and allocation pattern were similar. At the first harvest, the crops had taken up *ca.* 2.4–3.6% of added Cd. At the third harvest, the uptake was between 6.9 and 13.0%. It was not possible to identify any correlations between the percentage taken up and the Cd-concentration in the soil or crop species.

For both species the Cd uptake increased in absolute terms with higher concentration in the growth medium and there were significant differences between the treatments as shown by different letters above the columns. No significant difference between the species was found either at the first or at the third harvest. The allocation pattern of willow was different from those of the wheat cultivars. Willow stored most of the Cd aboveground, as indicated by a root:shoot ratio below 1.00 (Table IV in Appendix) at both the first and the third harvest. Both Dragon and Vinjett stored large amounts in the roots (Tables V-IV in Appendix) and the Cd concentrations were highest in the roots, too. The transport of Cd to the shoot increased as the plants grew larger, as indicated by the decrease in the root:shoot ratio at the third harvest. Willow had the highest amounts of Cd in the leaves and lowest in the stem, but the Cd concentration was highest in the stems. The relationship between the concentrations in the root and that in the shoot varied throughout the treatments and harvests.

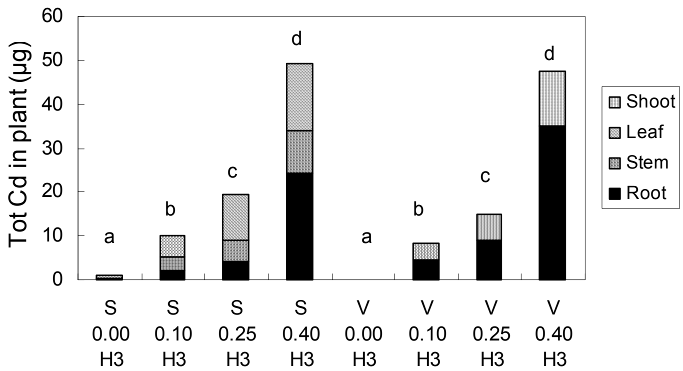

Figure 5. The total amount of Cd taken up as well as allocation to different plant parts of Salix viminalis and wheat cv Vinjett. Willow (S) was divided into root, stem and leaves and Vinjett (V) was divided into root and shoot. The values are the average values from the four treatments 0.00, 0.10, 0.25 and 0.40µg g^{-1} and different letters above the columns show the significance.

No significant correlation was found between root length and the amount of Cd, and the Cd concentration of the plants (results not shown). The small number of replications made this result hard to evaluate. A tendency was found in Dragon that the amount increased with increasing root length, but no conclusions could be drawn from this.

As mentioned above, the uptake of Cd did not depend on species, as is shown in Figure 5. The percentage of total added Cd taken up was similar for the different crops at harvest 1 and 3 respectively (Table IV-VI in Appendix). Use of the results from all crops as a basis for the statistical calculations, showed that the total amount of Cd in the plants from the first harvest was highly significantly different (***) between the 0.10 and 0.40 treatments (results not shown). At the third harvest there were highly significantly differences (***) between the treatments, except for treatments 0.10 and 0.25 (**). When the statistical analysis was made for each plant species, the significance of differences between the Cd concentrations were more difficult to demonstrate; there were too few degrees of freedom (results not shown).

A test of significant variations of the amount of Cd in plants at first and third harvests showed that there were interactions between the treatments and harvests, *i.e.* time of growth. Figure 6a-c shows the relationship between the amount of Cd in the three crops and the Cd concentration in the soil solution at the first and the third harvest respectively. The uptake had increased to the third harvest for the three treatments with Cd applications. However, the amount of Cd in the plant at the third harvest was sometimes similar to the amount of Cd in another treatment at the first harvest, *e.g.* the 0.10 treatment of the third harvest had the same amount of Cd as the 0.40 treatment at the first harvest.

Pairwise comparisons of treatment and harvest were made to search for significant differences between certain values, *e.g.* the 0.10 treatment at the first harvest (H1 0.10) compared to the 0.10 treatment at the third harvest (H3 0.10). For willow and Dragon, the result (not shown) was that only the 0.40 treatment at the third harvest was significantly different (***) from the other. Vinjett had significant differences between all of the treatments at both the first (*) and the third (***) harvest. Interactions between harvest and treatment were also found when the amount of Cd in root and shoot of wheat and in stem, leaf and root of willow were compared (results not shown). The comparison of H1 0.4 and H3 0.10 was not significant, neither for species nor for plant part, while H3 0.40 was significantly different (***) from all of the other values. An exception was Vinjett, which had significant differences in all the pairwise comparisons when the values for root and shoot were considered.

The uptake of Cd increased as the concentration of both growth medium (Fig. 7) and soil solution (Fig. 8), respectively, increased, giving R^2-values between 0.57 and 0.93. The uptake of Cd was proportional to the Cd concentration of the soil solution too, when the first (H1) and third (H3) harvests were considered individually (Fig. 6a-c).

Comparison of the total amount of Cd in the plant and total plant biomass of all treatments from both the first and the third harvest (Fig. 9), revealed no R^2-values greater than 0.30, the value chosen here to set the lower limit for an adequate correlation. When the plant uptake at the first and third harvests was considered separately, some of the R^2–values were above 0.30 (results not shown in figure). The R^2-values for Dragon and Vinjett were 0.35 and 0.49, respectively, at the first harvest, and at the third harvest only Vinjett showed correlations between the total amount of Cd in the plant and plant DM (R^2= 0.51). Correlation between the total amount of Cd in the plant for all treatments and root DM was found only for Vinjett (R^2 = 0.34), when values from both H1 and H3 were used. For Vinjett, the R^2 -value for the treatments at the first harvest was 0.65, and that from the third harvest was 0.60 (not shown).

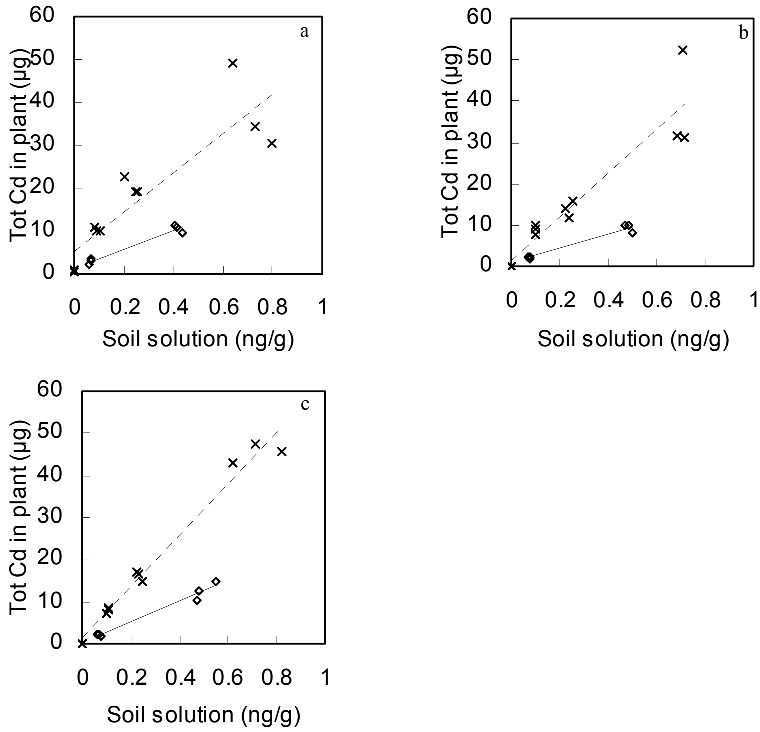

Figure 6a-c. Relationship between the total amount of Cd in plants and the concentration in soil solution as a function of time for (a) Willow (H1 R^2= 0.96, H3 R^2= 0.78), (b) Dragon (H1R^2= 0.95, H3 R^2= 0.88) and (c) Vinjett (H1R^2= 0.98, H3 R^2= 0.98). ◊——◊ Harvest 1 (H1) and ×----× Harvest 3 (H3).

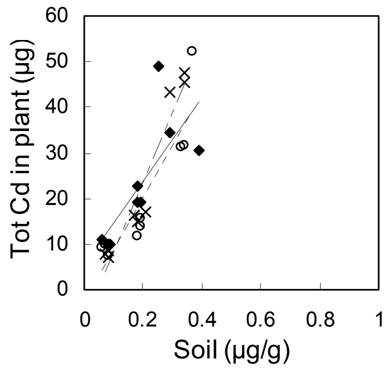

Figure 7. Relationship between the total amount of Cd in plants and the concentration in growth medium. Values from first and third harvest. ♦——♦ Willow R^2= 0.57, ○----○ Dragon R^2= 0.82, and ×-——-× Vinjett, R^2= 0.93.

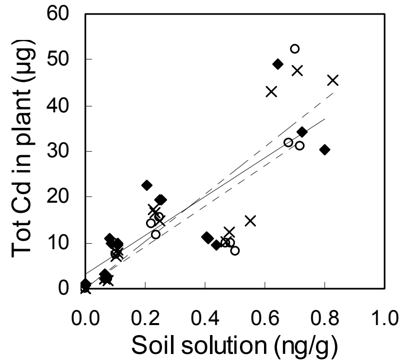

Figure 8. Relationship between the total amount of Cd in plants and the concentration in soil solution. Values from first and third harvest. ♦——♦ Willow R^2= 0.68, ○----○ Dragon R^2= 0.69 and ×-–—-× Vinjett, R^2= 0.79.

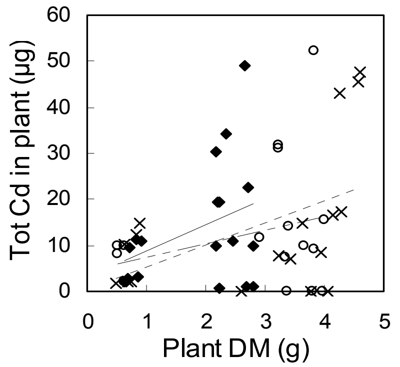

Figure 9. Amount of Cd in plant compared with the dry mass of the plant from all treatments at the first harvest and third harvest. ♦——♦ Willow R^2= 0.13, ○----○ Dragon R^2= 0.10 and ×-–—-× Vinjett, R^2= 0.25.

Very high correlations were found between the amount of Cd in the plant and the DM of the plant (Fig. 10a-b) and root (Fig. 11a-b), when results from H1 and H3 of the 0.10 and 0.40 treatment were used. The two wheat cultivars had similar plant and root biomasses, as well as Cd uptake within each treatment. Their plant and root biomass was greater than that of willow in both treatments, which led to higher Cd content per unit of dry mass, in willow.

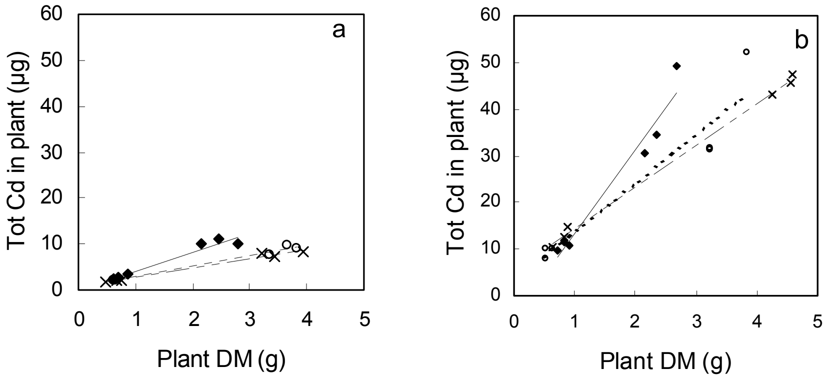

Figure 10a-b. Total amount of Cd in plant compared with the dry mass of the whole plant at treatments (a) 0.10 µg g^{-1} and (b) 0.40 µg g^{-1} at the first and third harvest. ♦——♦ Willow (a) R^2= 0.95, (b) R^2= 0.95, ○----○ Dragon (a) R^2= 0.98, (b) R^2= 0.91 and ×-——-× Vinjett, (a) R^2= 0.98, (b) R^2= 0.998.

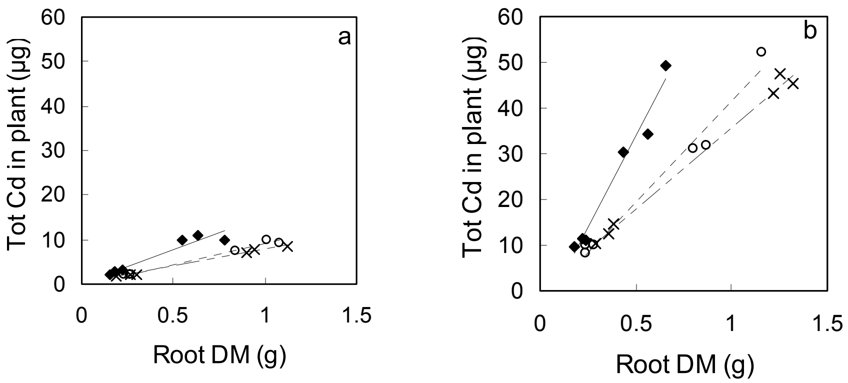

Figure 11a-b. Total amount of Cd in plant compared with the dry mass of the root at treatments (a) 0.10 µg g^{-1} and (b) 0.40 µg g^{-1} at the first and third harvest. ♦——♦ Willow (a) R^2= 0.91, (b) R^2= 0.97, ○----○ Dragon (a) R^2= 0.99, (b) R^2= 0.98 and ×-——-× Vinjett, (a and b) R^2= 0.99.

The total amount of Cd in the plant was not proportional to the root length of willow and Vinjett. Only Dragon had an R^2 of 0.44, which indicates a proportional correlation between Cd-uptake and root length. The Cd concentration in the entire plant, roots or the above ground parts was not proportional to the root length, neither was the amount of Cd in the root (results not shown).

Discussion

Results from the experiment revealed that the uptake of Cd in plants was proportional to the concentration of Cd in both soil solution and soil (Fig. 7-8). Plants grown at higher Cd concentrations in soil solution took up larger amounts of Cd than those grown at low concentrations. The statement supports the linear phase at low concentrations of Michaelis-Menten equation (Eq. 1), which assumes that the uptake rate is associated with the concentration in the growth medium. The uptake function for higher concentrations close to saturation was not tested in the present study. The time aspect also influenced the amount of Cd that plants take up (Figure 6a-c). Plants grown at low soil Cd concentration for a long period may take up the same amount of Cd as plants grown at high soil Cd concentration for a shorter period.

Growth

The plants did not use all the added nutrients, except willow at the first harvest (Tab. I-III in Appendix), which explains why the relative growth rates (R_G) were lower than the relative addition rate (R_A). Probably, the (R_A) was overestimated for both species, since parameters such as soil resistance and genetic variation may contribute to the reduction of growth compared to the addition rate. Nevertheless, the plants did not suffer from nutrient deficiency, which could otherwise have affected growth. Neither did we expect the growth or uptake to be disturbed by too high salt concentrations in the growth medium, since the soil *p*H stayed at neutral values. The N uptake varied only slightly between the four treatments, hence it was concluded that the amount of Cd in the growth medium did not affect the plants, since concentrations below the toxic level were used.

The value for relative growth rate had to be assumed, since it was not the same plant that was weighed repeatedly, but plants from the same treatment that were grown under the same conditions. The dry mass at the start of nutrient addition was based on the assumption that dry matter was 10% of fresh biomass; therefore, the values of for St-H1 and St-H3 may be incorrect. The R_G of willow between the harvests was generally lower than the addition rate (Table 4). An explanation of this may be that the resistance of the substrate made it more difficult for roots to develop, compared to growth in nutrient solution, from which the value of R_A was taken. Both Dragon and Vinjett grew at an R_G close to R_A between the first and the third harvest, which indicates a steady state, *i.e.* plants grew at a constant rate. However, some of the treatments grew faster between the first and the second harvest, and slowed down to the third, and *vice versa*. This was a result of the plants' adjusting to the amount of nutrient supplied. If they grew slowly at the beginning, they could grow more quickly when the supply of nutrient remained at a steady addition rate. The plants adjust to the growth conditions, and settle in to a steady state after some time.

The roots of wheat decreased as a percentage of total plant mass as they grew older. This pattern is typical of annual crops, which can be compared to the willow, which is a perennial and grow vegetative for a long period before flowering. Generally, the wheat plants were shorter than those grown in agricultural fields. Field-grown plants normally have a height of 1 m at harvest, whereas Dragon in this experiment attained a height of *ca*. 41–55 cm and Vinjett *ca*. 52–67 cm at the third harvest. One explanation for this may be that these plants grew with more space than field-grown plants, and did not need to elongate to achieve a better light supply. At the end of the experiment, the wheat cultivars were not yet mature, but had began heading (DC 43–55). If the experiment had continued, the plants probably would have elongated more as they matured. A further difference from field-grown wheat was that these plants were fertilised in small doses during a long period, to develop a steady state, whereas in the field, fertilisers are supplied once or twice per season. Micronutrients are mostly taken from the soil store or are added as supplements. A combination of diversity in light and nutrient supply may explain the different growth habit as compared to field-grown wheat.

Uptake of cadmium

Both species took up cadmium if the growth medium contained Cd. Plants of willow grown in Cd-free soil did, however, contain Cd at analysis. The most plausible explanation is that the cuttings contained a small amount of Cd, since they were taken from an outdoor plantation. As the plants grew larger, they allocated Cd from the cuttings to roots and shoot.

The results showed that the plant uptake of Cd was regulated by the amount of Cd in the substrate, since the amount of Cd in the plants increased as the concentration in the soil increased. Eriksson (1990) found that Cd uptake in wheat depended on the amount of plant-available Cd in the soil, and Gerritse (1983) related the uptake to the concentration of exchangeable Cd. Östman (1996), however, saw no connection between the Cd uptake of field-grown *Salix*-clone 78183 and the Cd concentration in soil that ranged from 0.13 $\mu g\ g^{-1}$ dry soil to 0.68 $\mu g\ g^{-1}$ dry soil. These values of soil concentration are in the same range as the values in the present experiment. Neither did Del Castillo & Chardon (1995), who investigated the uptake of Cd in endive (*Cichorium endiva* L.), spinach (*Spinacia oleracea* L.) and lettuce (*Lactuca sativa* L.), find any correlation between Cd concentrations in soil and the leafy parts of the three species.

Plants grown at high Cd concentration in soil for a long time in this experiment had taken up the highest amounts of Cd, compared to plants grown at the lower concentrations and for shorter periods (Fig. 6a-c). The uptake of Cd was proportional to the biomass of both the aboveground plant and the roots (Figure 10a-b and Figure 11a-b), when plants grown at the same Cd concentration were considered. Cd uptake increased at the same rate as the increase in biomass and hence, the concentration remained almost unchanged (Tab. IV-VI). Göransson (1999) and Greger *et al.* (2002) found results for *Salix* which support the results of the present study, and Greger *et al.* (2003) concluded that Cd uptake depends on biomass production, which in turn depends on nitrogen availability. The results shown in Figures 10 and 11 indicates that willow may be more efficient at taking up Cd than wheat, since the uptake per unit of biomass was higher for *Salix viminalis*. However, no conclusions on the driving force for the more efficient uptake could be drawn from the results in this study.

The Cd concentration in the soil solution of the 0.40 treatments at the third harvest appeared to be high compared to the concentration in the growth medium in relation to the treatments 0.10 and 0.25 μg Cd g^{-1} dry soil (Fig 3). This phenomenon should be looked upon in relation to the availability of Cd. Cd may adsorb or dissolve due to soil characteristics. Soil *p*H had probably little impact on this, since it was neutral in all samples of soil solution. There was no organic matter in the substrate that could adsorb Cd, so that aspect of adsorption may be disregarded. However, the binding sites on the substrate particles, *e.g.* vermiculite, may have played an important part in adsorbing or dissolving Cd or both. The concentration in the soil solution may be considered as a Langmuir isotherm (see Background). In the present experiment, there was equilibrium between the concentrations in the soil and in the soil solution in the treatments. An explanation to the higher concentration in the 0.40 treatment might be that there was less vermiculite in the soil of that treatment, and that it may have had a lower CEC, which resulted in another equilibrium constant, and that more Cd ions remained in the soluble phase and contributed to the high concentration in the soil solution.

The disease on the wheat cultivars, caused by the fungus *Drechslera tritici-repentis,* led to symptoms such as chlorosis and brown leaf tips, which inhibit photosynthesis. Some of the wheat plants were also infected by *Bipolaris sorokiniana,* which makes the stalk base brown, and after a while fungal hyphae enter the stele and grow down into the root. This may lead to a water and nutrient deficit, and to inhibition of the allocation of carbohydrates to the root. The diseases may have affected the growth and Cd uptake of Dragon, but only a minority of the plants of Vinjett, which may explain why Dragon grew less than Vinjett. This could also be the reason why Dragon had a tendency to take up less Cd than Vinjett, although but it was not shown statistically. Overall, significant results were more easily found for Vinjett than for Dragon, which may be due to the disturbed growth for Dragon.

Allocation

In the present experiment, the two wheat cultivars stored most of the Cd in the roots. Verkleij & Schat (1990) found that Cd is immobilised in root tissue after uptake, mostly as adsorbed Cd in AFS, where cation exchange and complex binding at the cell wall takes place. The root:shoot ratio of dry matter (Tables II-III) and the root:shoot ratio of the amount of Cd (Tables V-VI) were not identical, showing that the proportions of biomass and the amount of Cd were not the same in the different plant parts. The root:shoot ratios of both biomass and amount of Cd decreased between the first and the third harvest. This may be due to a larger transport of Cd within the plant, to growth of larger shoot and spikes, or both.

The root:shoot ratio of biomass of *S. viminalis* was slightly higher than the root:shoot ratio of the amount of Cd (Tables I & IV), which indicated that the relative allocation of Cd to the shoot was larger than the biomass allocation. At the third harvest, the amounts of Cd had increased in the shoot compared to the amount in the shoot at first harvest, and indicate an increase in transport of Cd up to the shoot. According to Landberg and Greger (1994), the uptake of Cd in *S. viminalis* clone 78183, compared to other clones of *Salix*, is high to medium, and the transport is low when it is grown in nutrient solution containing 1 µM Cd. Those plants were not fully supplied with nutrients, only with N, Ca and Cl, which may have affected uptake in the way that the plants took up any available ions, and may therefore not simulate the normal uptake of Cd. In this experiment the plants were given a full supply of nutrients, which made them grow optimally.

In the present experiment, the amount of Cd in willow was higher in the leaves than in stem or roots. The concentrations were, however, highest in the stems, except for treatment 0.40 at H1 and H3, where the concentrations in the roots were highest. This may be due to the high concentration of available Cd in the soil solution, which the plants took up and stored in the root. Klang-Westin and Perttu (2002) found that the amount of Cd was largest in the stem of 2-year-old plants, while the concentration was higher in the leaves than in the stem. The high amount of Cd in leaves has concerned researchers, because it may lead to redistribution of Cd to the topsoil at leaf-fall (Christensson & Tjell, 1983; Ericson, 1994, Greger & Landberg, 1995). Cd is taken up from the subsoil, and may accumulate in the topsoil, when residual biomass or leaves containing accumulated Cd are decomposed. Soil tillage may contribute to a mixing of Cd in the soil. A subsequent crop may therefore be influenced in its Cd uptake due to the increased amount of Cd in the surface soil layer.

Conclusions

Plants took up Cd to a larger extent at higher concentrations of Cd in the soil and soil solution. Time of growth influenced the total amounts taken up, since plants grown at high concentration for a short time might take up the same amount of Cd as plants grown at low concentration during a long time of period. An increase in total plant or in root biomass increased the uptake of Cd when grown at constant Cd concentrations in the soil.

Further research

Analysis of the remaining treatments may give more results on the correlation between the uptake of Cd and *(a)* the concentration in the soil and soil solution, and *(b)* growth pattern. The question of how time will affect uptake must be explored. Is it a question of how long a period of time for which the plant grows, that affects uptake, or is it an aspect of crop development? Does uptake in wheat remain linear at the end of the growing season, or does it stagnate as the plant matures? More comparative experiments with different crops may show which crop removes the largest amount of Cd during a certain period of time.

References

Andersson, A. & Bingefors, S. 1985. Trends and annual variations in Cd concentration in grain of winter wheat. *Acta Agriculture Scandinavia, 35*. 339-344.

Andersson, A., Nilsson, Å. & Håkansson, L. 1991. Metal concentration of the mor layer. *Swedish Environmental Protection Agency*, *Report 3990*. ISBN 91-620-3990-3. ISSN 0282-7298.

Blombäck, K. 2002. Modellansats för simulering av kadmiumdynamik i Salixodlingar. In: Perttu, K., Eriksson, J., Greger, M., Göransson, A., Blombäck, K., Klang-Westin. E. & Landberg, T. 2002. *Flöden och förråd av kadmium i systemet mark–Salix.* (In Swedish.) In press.

Cerealia. 2003. Vårt miljöarbete, Råvaror. (In Swedish.) http://www.dominoplaza.com/cerealia2/site.nsf/0/710C6D0DCFFA384AC1256A5C0032801B?OpenDocument (Accessed 20-Feb-03).

Cristensson, T.H. & Tjell, J.C. 1983. Interpretation of experimental results on cadmium crop uptake from sewage sludge amended soil. *Processing and Use of Sewage Sludge. Proc. Third International Symposium*. 358-369. Brighton

Del Castillo, P. & Chardon, W.J. 1995. Uptake of soil cadmium by three field crops and its prediction by a pH-dependent Freundlich sorption model. *Plant and Soil, 171*. 263-266.

Dickinsson, N.M., Punshon, T., Hodkinson, R.B. & Lepp, N.W. 1994. Metal tolerance and accumulation in willows. In: Aronsson, P. & Perttu, K. Willow vegetation filters for municipal wastewaters and sludges. A biological purification system. *Swedish University of Agricultural Science, Department of Ecology and Environmental research, Section of Short Rotation Forestry, Report, 50*. ISBN 91-576-4916-2. ISSN 0282-6267.

Ericson, S.O. 1994. *Salix* can remove cadmium from arable land—technical and infrastructural implications. In: Aronsson, P. & Perttu, K. Willow vegetation filters for municipal wastewaters and sludges —A biological purification system. *Swedish University of Agricultural Science, Department of Ecology and Environmental research, Section of Short Rotation Forestry, Report, 50*. ISBN 91-576-4916-2. ISSN 0282-6267.

Ericson, T. 1995. Growth and shoot: root ratio of seedlings in relation to nutrient availability. *Plant and Soil, 168-169*. 205-214.

Eriksson, J.E. 1990. Factors influencing adsorption and plant uptake of Cd from agricultural soils. *Swedish University of Agricultural Science, Department of Soil Science, Reports and Dissertations, 4*. ISBN 91-576-4111-0. ISSN 1100-4525.

Eriksson, J., Andersson, A. & Andersson, R. 1997. Tillståndet i svensk åkermark. *Naturvårdsverket förlag*, *Report 4778*. ISBN 91-620-4778-7. ISSN 0282-7298. (In Swedish with English summary.)

Eriksson, J., Öborn, I., Jansson, G. & Andersson, A. 1996. Factors Influencing Cd-content in crops. *Swedish Journal of agricultural research, 26*. 125-133.

European Commission (2001). *Commission Regulation (EC) No 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. (Text with EEA relevance.)*

Göranson, A. 1999. Är kadmiumupptaget i *Salix* beroende av biomassatillväxt och/eller vattenflöden? In: Göransson, A. (Ed.) Kadmium i jordbrukssamhället – Ger odling av *Salix* en möjlighet att minska som kadmiumbelastningen? *Swedish University of Agricultural Science, Department of Short Rotation Forestry, Report, 65*. 57-60. ISBN 91-576-5684-3. ISSN 1402-6910 (In Swedish.)

Greger, M., Göransson, A., Klang-Westin, E., Landberg, T. & Perttu, K. 2002. Analys av biomassaproduktionens inverkan på kadmiumupptag och kadmiumbortförsel. In: Perttu, K., Eriksson, J., Greger, M., Göransson, A., Blombäck, K., Klang-Westin. E. & Landberg, T. 2002. Flöden och förråd av kadmium i systemet mark. (In Swedish.) In press.

Greger, M. & Landberg, T. 1995. Analys av kadmiumhalten i *Salix* relaterat till kadmiumhalten i jorden. *Rapport från Vattenfall Utveckling AB, 1995:9*. ISSN 1100-5130. (In Swedish.)

Greger, M. & Landberg, T. 1996. Kadmiumupptag och tolerans hos olika *Salix*kloner, skillnader som möjliggör olika användningsområden. In: Göransson, A. (Ed.) *Salix* som kadmiumfilter. *Swedish University of Agricultural Science, Department of Ecology and Environmental research, Section of Short Rotation Forestry, Report, 55*. 15-28. ISBN 91-576-5110-8. ISSN 0282-6267. (In Swedish.)

Greger, M., Landberg, T. & Bengtsson, L. 2003. Cadmium uptake in wheat—influence of nitrogen and nitrogen supplementation. In: Ivarsson, K. & Öborn, I. (Eds). Cadmium from Plough to Plate. Report from Cadmium Seminar on 12 June 2002 in Uppsala, Sweden. *Swedish University of Agricultural Science, Swedish Cadmium Network. Report FOOD 21, 5.* 17. ISBN 91-576-6279-7. ISSN 1650-5611.

Haghiri, F. 1974. Plant uptake of cadmium as influenced by cation exchange capacity, organic matter, zinc and soil temperature. *Journal of Environmental Quality 3,* 180-183.

Hardiman, R.T. & Jacoby, B. 1984. Adsorption and translocation of Cd in bush bean (*Phaseolus vulgaris*). *Physiologia Plantarum, 61*. 670-674.

Hydroagri. 2003. Vårt miljöprogram—Vill du veta mer http://www.hydroagri.se/. (Accessed 18-Mar-03). (In Swedish.)

Ingestad, T. & Lund, A-B. 1986. Theory and Techniques for Steady State Mineral Nutrition and Growth of Plants. *Scandinavian Journal of Forest Research, 1*. 439-453.

Kabata-Pendias, A. & Pendias, H. 1992. *Trace elements in soils and plant.* 2nd ed . CRC Press inc., Boca Raton, FL, USA. 365p.

Klang-Westin, E. & Perttu, K. 2002. Effects of nutrient supply and soil cadmium concentration on cadmium removal by willow. *Biomass and Bioenergy, 6.* 415-426

Koeppe, D.E. 1977. The uptake, disrtibution and effect of cadmium and lead in plants. *The Science of The Total Environment, 7*. 197-206.

Landberg, T. & Greger, M. 1994. Can heavy metal tolerant clones of *Salix* be used as vegetation filters on heavy metal contaminated land? In: Aronsson, P. & Perttu, K. Willow vegetation filters for municipal wastewaters and sludges —A biological purification system. *Swedish University of Agricultural Science, Department of Ecology and Environmental research, Section of Short Rotation Forestry, Report, 50*. ISBN 91-576-4916-2. ISSN 0282-6267.

Lantbrukarnas Riksförbund. 2003. *Säkert kretslopp av växtnäring mellan stad och land.* http://www.lrf.se/miljo/svenskamodellen/miljohansyn.htm#vaxtnaring . (Accessed 04-Feb-03). (In Swedish.)

Larcher, W. 1995. *Physiological Plant ecology: ecophysiology and stress physiology of function groups*. 3rd edition. Springer. Berlin. 506 pp. ISBN 3-540-58116-2.

Levi-Minzi, R., Soldatini, G.F. & Riffaldi, R. 1976. Cadmium adsorption by soils. *Journal of Soil Science 27,* 10-15.

Marschner, H. 1995. *Mineral Nutrition of Higher Plants*. 2nd edition. Academic Press. London. 889 pp.

McBride, M.B. 1995. Toxic and accumulation from agricultural use of sludge: Are USEPA regulations protective? *Journal of Environmental Quality 24*. 5-18.

Mengel, K. & Kirkby, E A. 1982. *Principles of Plant Nutrition.* 3rd edition. International Potash Institute. Worblaufen-Bern. 655 pp.

Ministry of Social Affairs, Public Health and the Environment. 1999. Risk assessment—Cadmium oxide. Part 1. *Belgian Federal Department of the Environment. Brussels.*

Nationalencykopledin. 1993a. *Kadmium, volym 10*. 326-327. Höganäs. (In Swedish.)

Nationalencykopledin. 1993b. *Langmuir adsorptionsisoterm*, *volym12*. 111. Höganäs. (In Swedish.)

Naturvårdsverket. 2001. *Naturvårdsverketsföreskrift SNFS 2001:5. Ändring i kungörelse SNFS 1994:2.* (In Swedish.)

Öborn, I., Janson, G. & Johnsson, L. 1995. A field study on the influence of soil pH on trace elements levels in Spring wheat (*Triticum aestivum*), potatoes (*Solanum tuberosum*) and carrots (*Daucus carota*). *Water Air and Soil Pollution, 85*. 835-840.

Östman, G. 1996. *Salix* förmåga att ta upp kadmium—en fältstudie. *Swedish University of Agricultural Science, Department of Ecology and Environmental research, Section of Short Rotation Forestry, Report, 55*. 71-73. ISSN 0282-6267. ISBN 91-576-5110-8. (In Swedish.)

Palm, V. 1996. Modellering av kadmiumupptag i *Salix*. In: Göransson, A. (Ed.) *Salix* som kadmiumfilter. *Swedish University of Agricultural Science, Department of Ecology and Environmental research, Section of Short Rotation Forestry, Report, 55*. 71-73. ISSN 0282-6267. ISBN 91-576-5110-8. (In Swedish.)
Salisbury, F.B. & Ross, C.W. 1992. *Plant Physiology*. 4th edition. Wadsworth. Belmont, CA. 682 pp.
SAS Institute Inc. 1987. *SAS/STAT™ guide for Personal Computers.* Version 6th edition. SAS Institute Inc. Cary, NC. 1028 pp.
Singh, B.R. & McLaughlin, M.L. 1999. Cadmium in Soils and Plants. In: McLaughlin, M.L. & Singh, B.R. (Eds.). *Cadmium in Soils and Plants*. Kluwer Academic Press. Dordrecht, The Netherlands. 271 pp.
Svenskt Sigill. 2003. Bakgrundsinformation. http://www.svensktsigill.com. (Accessed 04-Feb-03). (In Swedish.)
Taiz, L. & Zeiger, E. 1998. *Plant Physiology*. 2nd edition. Sinauer Associates. Sunderland, MA. 792 pp.
Verkleij, J.A.C. & Schat, H. 1990. Mechanisms of metal tolerance in higher plants, pp. 179-191. In: Shaw, A.J. (ed). *Heavy Metal Tolerance in Plants; Evolutionary Aspects.* CRC Press, Inc., Boca Raton, FL. 355 pp.
Verwijst, T. 1996. Cyclic and progressive changes in short-rotation willow coppice systems. *Biomass and Bioenergy 2*. 161-165.
Zadoks, J.C., Chang, T.T & Konzak, C.F. 1974. A decimal code for the growth stages of cereals. *Weed Research, 14*. 415-421.

Acknowledgements

This work was financed by the union Agrifack (Foundation of Axel Adler) and The Royal Swedish Academy of Agriculture and Forestry (KSLA).

The author wishes to thank:

• The supervisors Dr Karin Blombäck, Dep. of Soil Sciences, and Dr. Anders Göransson, Dep. of Production Ecology, for their knowledge and inspiration during the process;

• Ingrid Öborn, Dep. of Soil Sciences, for acting as examiner;

• Monika Kähr for your expertise and helping out with cultivation and harvest of the plants;

• Eira Casenberg for support and analyses of the samples;

• Jeremy Flower-Ellis for helping with language and layout;

• Staff at SPEK (Department for Production Ecology) and Department of Soil Sciences for their scientific and social support;

• Niclas and Torgny for everything.

Appendix

Table I. *Average results of biomass growth from the harvests of Salix viminalis grown in four Cd treatments; 0.00, 0.10, 0.25 and 0.40 $\mu g\ g^{-1}$ dry soil. Plt = plant, St = stem, L = leaves, Rt = root, DM = dry matter, Rt/(St+L) = root shoot index, Rt lgth (m) = root length and % of added N = N found in plant in percentage of added*

Treatment	Harvest 1				Harvest 2				Harvest 3			
	0.00	0.10	0.25	0.40	0.00	0.10	0.25	0.40	0.00	0.10	0.25	0.40
Plt DM (g)	0.81	0.73	0.76	0.82	1.46	1.46	1.39	1.28	2.56	2.47	2.39	2.39
St DM (g)	0.15	0.13	0.14	0.14	0.32	0.31	0.30	0.24	0.64	0.61	0.57	0.61
L DM (g)	0.46	0.41	0.44	0.46	0.74	0.75	0.71	0.70	1.24	1.21	1.21	1.23
Rt DM (g)	0.20	0.19	0.19	0.21	0.41	0.40	0.35	0.33	0.90	0.65	0.60	0.55
Rt/(St+L)	0.32	0.35	0.30	0.35	0.38	0.37	0.34	0.35	0.36	0.36	0.34	0.30
Rt lgth (m)	-	-	24.8	-	-	-	47.1	-	-	-	67.7	-
% added N	-	95.7	-	100	-	-	-	-	70.8	68.3	68.9	61.7

Table II. *Average results of biomass growth from the harvests of spring wheat cultivar Dragon grown in four Cd treatments; 0.00, 0.10, 0.25 and 0.40 $\mu g\ g^{-1}$ dry soil. Plt = plant, Sh = shoot,Rt = root, DM = dry matter, Rt/Sh = root shoot index, Rt lgth (m) = root length and % of added N = nitrogen found in plant in percentage of added*

Treatment	Harvest 1				Harvest 2				Harvest 3			
	0.00	0.10	0.25	0.40	0.00	0.10	0.25	0.40	0.00	0.10	0.25	0.40
Plt DM (g)	0.62	0.63	0.66	0.55	1.54	1.21	1.38	1.55	3.72	3.61	3.44	3.43
Sh DM (g)	0.33	0.38	0.38	0.30	0.97	0.80	0.93	0.93	2.65	2.63	2.62	2.49
Rt DM (g)	0.29	0.25	0.29	0.26	0.57	0.41	0.45	0.62	1.07	0.98	0.83	0.94
Rt/Sh	0.87	0.67	0.76	0.84	0.59	0.51	0.48	0.67	0.40	0.37	0.32	0.38
Rt lgth (m)	-	-	27.6	-	-	-	47.4	-	-	-	99.2	-
% added N	-	32.8	-	35.8	-	-	-	-	66.8	66.3	61.6	62.7

Table III. *Average results of biomass growth from the harvests of spring wheat cultivar Vinjett grown in four Cd treatments; 0.00, 0.10, 0.25 and 0.40 $\mu g\ g^{-1}$ dry soil. Plt = plant, Sh = shoot, Rt = root, DM = dry matter, Rt/Sh = root shoot index, Rt lgth (m) = root length and % of added N = nitrogen found in plant in percentage of added*

Treatment	Harvest 1				Harvest 2				Harvest 3			
	0.00	0.10	0.25	0.40	0.00	0.10	0.25	0.40	0.00	0.10	0.25	0.40
Plt DM (g)	0.73	0.63	0.70	0.78	1.80	1.77	1.85	1.96	3.48	3.53	4.02	4.47
Sh DM (g)	0.42	0.38	0.43	0.44	1.10	1.14	1.27	1.21	2.58	2.54	3.08	3.21
Rt DM (g)	0.31	0.25	0.27	0.34	0.70	0.62	0.58	0.75	0.90	0.99	0.94	1.27
Rt/Sh	0.74	0.67	0.64	0.78	0.63	0.54	0.46	0.62	0.35	0.39	0.31	0.39
Rt lgth (m)	-	-	29.7	-	-	-	54.6	-	-	-	108	-
% added N	-	47.8	-	62.7	-	-	-	-	62.5	61.0	65.1	69.9

Table IV. *Amount of cadmium and cadmium concentrations (mean and standard deviation, n=3) of Salix viminalis grown in four Cd treatments; 0.00, 0.10, 0.25 and 0.40 µg g^{-1} dry soil. Plt Cd (µg/g)= concentration of Cd in plant, Plt Cd (µg) = total amount of Cd in plant, St =stem, L = leaves, Rt = root, Rt/(St+L)= root shoot index (µg $µg^{-1}$)*

Treatment	Harvest 1		Harvest 3			
	0.10	0.40	0.00	0.10	0.25	0.40
Plt Cd (µg)	2.84± 0.51	10.6± 0.94	0.92± 0.25	10.3± 0.62	20.4± 1.96	38.0± 9.82
St Cd (µg)	0.66± 0.13	2.04± 0.32	0.21± 0.06	2.79± 0.30	5.41± 0.04	8.61± 1.27
L Cd (µg)	1.41± 0.25	3.37± 0.17	0.54± 0.13	5.22± 0.53	10.4± 0.17	12.5± 2.27
Rt Cd (µg)	0.78± 0.13	5.22± 0.47	0.18± 0.07	2.27± 0.13	4.63± 0.81	16.9± 6.43
St Cd (µg/g)	5.24± 0.41	14.2± 1.22	0.33± 0.01	4.60± 0.65	9.52± 0.40	14.1± 0.60
L Cd (µg/g)	3.40± 0.32	7.35± 0.68	0.43± 0.07	4.35± 0.58	8.64± 0.83	10.1± 1.09
Rt Cd (µg/g)	4.14± 0.24	24.7± 1.76	0.25± 0.09	3.55± 0.48	7.67± 0.79	29.9± 5.84
Rt/(St+L)	0.37± 0.01	0.97±0.00	0.22± 0.04	0.29± 0.04	0.29± 0.03	0.78± 0.17
% added Cd	3.3	3.0	-	12.1	10.2	10.8

Table V *Amount of cadmium and cadmium concentrations (mean and standard deviation, n=3) of wheat cv Dragon grown in four Cd treatments; 0.00, 0.10, 0.25 and 0.40 µg g^{-1} dry soil. Plt Cd (µg/g)= concentration of Cd in plant, Plt Cd (µg) = total amount of Cd in plant, Sh = shoot, Rt = root and Rt/Sh= root shoot index (µg $µg^{-1}$))*

Treatment	Harvest 1		Harvest 3			
	0.10	0.40	0.00	0.10	0.25	0.40
Plt Cd (µg)	2.06± 0.04	9.33± 1.11	0.00± 0.00	8.79± 1.16	13.8± 1.93	38.4±12.0
Sh Cd (µg)	0.44± 0.22	1.73± 0.29	0.00± 0.00	2.77± 0.07	4.93± 1.12	10.4± 1.42
Rt Cd (µg)	1.62± 0.05	7.60± 0.86	0.00± 0.00	6.02± 1.20	8.85± 0.82	27.9± 10.7
Plt Cd (µg/g)	3.25± 0.01	16.8± 1.91	0.00± 0.00	2.43± 0.21	4.00± 0.11	11.0± 2.24
Sh Cd (µg/g)	1.16± 0.64	5.71± 0.50	0.00± 0.00	1.05± 0.08	1.87± 0.14	4.19± 0.31
Rt Cd (µg/g)	6.39± 0.23	29.9± 3.53	0.00± 0.00	6.13± 0.70	10.8± 0.81	28.9± 4.94
Rt/Sh	3.70± 0.22	4.42± 0.37	-	2.18± 0.46	1.84± 0.30	2.63± 0.67
% added Cd	2.4	2.7	-	10.4	6.9	11.1

Table VI. *Amount of cadmium and cadmium concentrations (mean and standard deviation, n=3) of wheat cv Vinjett grown in four Cd treatments; 0.00, 0.10, 0.25 and 0.40 µg g^{-1} dry soil. Plt Cd (µg/g) = concentration of Cd in plant, Plt Cd (µg) = total amount of Cd in plant, Sh = shoot, Rt = root and Rt/Sh= root shoot index (µg $µg^{-1}$)*

Treatment	Harvest 1		Harvest 3			
	0.10	0.40	0.00	0.10	0.25	0.40
Plt Cd (µg)	2.04± 0.30	12.5± 2.17	0.00± 0.00	7.82± 0.60	16.2± 1.09	45.4± 2.17
Sh Cd (µg)	0.44± 0.02	2.12± 0.48	0.00± 0.00	3.17± 0.44	6.45± 0.50	12.0± 0.82
Rt Cd (µg)	1.60± 0.28	10.4± 1.70	0.00± 0.00	4.65± 0.44	9.74± 0.67	33.4± 1.42
Plt Cd (µg/g)	3.29± 0.28	16.0± 0.65	0.00± 0.00	2.22± 0.21	4.03± 0.08	10.2± 0.19
Sh Cd (µg/g)	1.21± 0.22	4.82± 0.40	0.00± 0.00	1.24± 0.07	2.09± 0.03	3.75± 0.09
Rt Cd (µg/g)	6.41± 0.48	30.3± 0.91	0.00± 0.00	4.73± 0.60	10.4± 0.39	26.4± 1.35
Rt/Sh	3.61± 0.50	4.93± 0.32	-	1.49± 0.25	1.51± 0.08	2.79± 0.11
% added Cd	2.4	3.6	-	9.2	8.1	13.0