Effects of Nitrogen Fertilization on the Cadmium Concentration in Winter Wheat Grain

Field studies on cadmium and nitrogen uptake and distribution in shoots as related to stage of development

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Abstract

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The thesis summarizes and discusses results from field studies on the effects of nitrogen (N) fertilization on cadmium (Cd) concentration in winter wheat (*Triticum aestivum* L.). It also deals with Cd and N uptake and distribution in shoots as related to stage of development. Furthermore, the possibility to predict the grain Cd concentration at harvest from the Cd concentration in the shoot at earlier stages of development was investigated. The studies were conducted in the major wheat-growing districts in southern and central Sweden.

It was shown that N fertilization caused an increase in Cd concentration in winter wheat grain, independent of site and cultivar. For an N application rate of 145 kg N ha^{-1} (Swedish norm) an increase of 30 kg (to increase grain protein content) would increase the grain Cd concentration by 6-14%. There were no significant effects of N application strategies such as earlier application and split rates on grain Cd concentration. The amounts of Cd and N in wheat shoots increased with time during the entire study period, from stem elongation to maturity. Nitrogen appeared to be redistributed to the ear/grain as soon as the ear emerged, whereas for Cd such redistribution only occurred close to maturity. There was a general correlation between Cd concentration in shoots and Cd concentration in mature grain. The correlation was strongest at ear formation, about two months before harvest. However, some deviations from the general pattern related to specific sites and cultivars make further investigations necessary before prediction of the Cd concentration in harvested grain becomes practicable.

Key words: cadmium, fertilization, grain, nitrogen, plant uptake, cadmium prediction, *Triticum aestivum*

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Appendix

Papers I and II

The present thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Wångstrand, H., Eriksson, J. & Öborn, I. 2005. Effect of nitrogen fertilizer application on cadmium concentration in grain of winter wheat. (Submitted to European Journal of Agronomy)

II. Eriksson, J., Wångstrand, H. & Öborn, I. 2005. Accumulation and distribution of Cd and N in winter wheat shoots during the growing season. Early prediction of Cd concentration in harvested grain. (Manuscript)

Introduction

Background

Cadmium (Cd) is a non-essential heavy metal that is toxic to humans and all other living organisms and that is relatively mobile in the soil-plant system. Cd pollution of the environment started about 100 years ago as a result of anthropogenic activities such as mining, industry, agriculture and waste disposal (Alloway, 1995). However, the contamination of soil, fertilizers and food by Cd first attracted attention in the early 1960s. Interest in Cd in plants and soils then became greater when serious damage to human health caused by Cd was discovered in the 1970s in Japan (McLaughlin & Singh, 1999). Industrial pollution of rice fields with Cd-rich waste water caused 'itai-itai' disease, which involved skeletal deformation and kidney dysfunction in some local families who consumed the rice. The Cd content in Swedish agricultural soils and crops has attracted increasing attention in the last few decades as a result of a growing awareness about environmental and food quality issues among both producers and consumers.

According to data from 1995, Cd was used in Sweden in Ni-Cd batteries (73 ton/year), stabilizers (20 ton/year), protective plating on steel (0.5 ton/year) and in pigment in artistic paints, glass and ceramics (0.5 ton/year) (Lohm *et al.*, 1996). The use of Cd in batteries increased drastically between 1980 and 1990, but has decreased since then (Hellstrand & Landner, 1998). The use of Cd in most other products has decreased considerably since 1975. Sources of soil contamination by Cd are atmospheric emissions (*e.g.* from smelters, metal industries, coal combustion and incineration of municipal solid waste), sewage sludge, industrially contaminated land (chemical factories, *etc.*), mine waste dumps, corrosion of galvanized metal, and phosphatic fertilizers (Alloway & Steinnes, 1999). About 50% of the Cd added to Swedish agricultural soils is deposited from the air (Notter, 1993). Calculations made by Andersson (1992) indicate that the average Cd content in Swedish agricultural surface soils increased by approximately 30% during the 20th Century.

The major threat to human health from Cd is due to its accumulation in the kidneys, where it can cause damage at high concentrations. This kidney damage can progress to a disturbed vitamin D and calcium metabolism which can cause brittle bone disease, osteoporosis (Alfvén *et al.*, 2000). Humans are exposed to Cd mainly through food, although tobacco smoking and occupational exposure to CdO fumes are also significant sources. Hellstrand & Landner (1998) calculated from available data that 43% of the Cd ingested with food in Sweden comes from wheat flour. Wheat accumulates more Cd than the other commonly grown cereals in Sweden (Eriksson *et al.*, 2000). To enable the Cd concentration in wheat and other crops to be reduced, it is important to minimize the Cd contamination of soil, but also to understand how Cd bioavailability is affected by different soil and management factors.

Soil factors affecting crop Cd concentration

The total Cd concentration in the soil is one of the major factors affecting the Cd concentration in the plant. A large proportion of the soil Cd is generally of natural origin, which makes the concentration in the soil dependent to a large extent on the rock from which the soil has developed. There is a correlation between the geological origin of the parent material and the Cd concentration in the soil (Eriksson, Söderström & Andersson, 1995). From a review of the literature, Kabata-Pendias (2001) concludes that the average Cd concentrations in surface soils lie between 0.06 and 1.1 mg kg^{-1} with a calculated worldwide mean of 0.53 mg kg^{-1}. In a survey of Swedish agricultural soils, the average Cd concentration in the topsoil was 0.23 mg kg^{-1} with a variation from 0.02 to 2.83 mg kg^{-1} (Eriksson, Andersson & Andersson, 1997).

Soil pH is the most important factor affecting the Cd availability in soil, because it strongly influences the solubility of Cd (Linnman *et al.*, 1973; Andersson & Nilsson, 1974; Williams & David, 1976; Garcia-Miragaya & Page, 1978; Tiller, Nayyar & Clayton, 1979; Eriksson, 1990; Öborn, Jansson & Johnson, 1995). The plant availability of Cd decreases with increasing pH because Cd binds more strongly to the soil as the charge density and the deprotonation of specific adsorption sites increase (Garcia-Miragaya & Page, 1978; Abd-Elfattah & Wada, 1981). The sorption of Cd is a rapid process. In one study, 95% of the sorption took place within 10 minutes and equilibrium was reached in one hour (Christensen, 1984). In the pH interval from 4 to 7.7, Christensen found that the sorption capacity of the soils studied increased by a factor of three for a pH increase of one unit. At pH 6 the soil had a very high affinity for Cd. The pH of the subsurface soil may also be important for the uptake of Cd, since about 20-35% of the Cd in the plant is taken from the subsurface soil (Johnsson *et al.*, 2000).

An increase in organic matter and clay results in a lower Cd uptake by plants. This is because organic matter and clay have the ability to bind Cd and make it less available to plants due to their high cation-exchange capacities, high densities of specific adsorption sites (clay) and metal-complexing ability (organic matter), resulting in less Cd in the soil solution (Haghiri, 1974; Stevenson, 1976; Ziper, Komarneni & Baker, 1988).

Relative excesses of Cu, Ni, Se, Mn and P can reduce the uptake of Cd by plants (Page, Bingham & Chang, 1981). Zn can also affect the Cd uptake, but it seems as though the interaction is concentration-dependent. Zn has an antagonistic effect on Cd uptake in soils with low Cd concentrations and either a synergistic or a zero effect in soils with high Cd concentrations (Page, Bingham & Chang, 1981). In an experiment in South Australia, applications of low rates of Zn (5 kg ha^{-1}) at sowing reduced Cd concentrations in wheat grain in areas with slight to severe Zn deficiency (Oliver et al., 1994). According to the authors, this effect could be due to rectification of the damage to root tissue caused by the Zn deficiency, as well as to competition between Cd and Zn for uptake. No further significant decreases in Cd concentration occurred at Zn application rates higher than 5 kg ha^{-1}. Welch *et. al.* (1999) reported that Zn has the potential to inhibit movement of Cd via the phloem in durum wheat. Providing adequate Zn levels may limit phloem loading of Cd into wheat grain.

Management factors affecting crop Cd concentration

There are several management factors affecting crop Cd concentration, *e.g.* the choice of plant species, cultivar and site as well as liming and fertilization.

Cd uptake differs very much between crop species. Cereals and legumes accumulate less Cd in the shoots than do leafy crops such as lettuce and spinach (Bingham *et al.*, 1975). In leafy vegetables, Cd accumulates in the leaves, whereas in cereals, accumulation is greatest in the roots and declines towards the top of the plant (Cutler & Rains, 1974). The Cd concentration of crops grown in solution culture increased in one investigation in the following order: oats, wheat < bean, pea, sunflower, cucumber < corn, mustard < radish, kale, rape < tomato, carrot < lettuce (Pettersson, 1977). Cultivars of durum wheat (*Triticum turgidum* L. var. *durum*) have been found to accumulate more Cd than those of common bread wheat (*Triticum aestivum* L.) at the same levels of soil Cd (Meyer *et al.*, 1982). For the most common species of cereal crops in Sweden, the Cd concentration in grain increases in the following order: barley < oats < wheat (Eriksson *et al.*, 2000).

The amount of Cd that enters the diet from a crop depends on how much Cd the crop accumulates, the quantities of the crop that are consumed and whether the Cd is accumulated in the edible part. The distribution of Cd in the plant differs depending on the plant species and growing conditions. Cadmium concentration has been found to be lower in grain than in straw of wheat, oats and corn (Grant *et al.*, 1999). In addition to uptake through the roots, Cd can be absorbed into foliage and translocated to other parts of the plant and this is a significant way for Cd to enter the food chain in areas where the atmospheric deposition is high (Alloway, 1995). Cadmium has been found to be bound to cytoplasmic proteins, so called phytochelatins, which usually contain cysteine. These proteins have been found in mushroom, bean, soybean, cabbage, wheat and other plant species (Alloway, 1995). Elevated concentrations of Cd in plant tissues may trigger the formation of phytochelatins, which could lead to retention of Cd in the roots. However, Stolt *et al.* (2003) could not find evidence supporting the hypothesis that differential grain Cd accumulation in spring bread wheat and durum wheat is due to differential phytochelatin-based Cd sequestration in the roots.

Cd accumulation may differ greatly among cultivars of the same species. Differences of 25-50% between cultivars have been shown for wheat and oats (Pettersson, 1977; Andersson & Pettersson, 1981; Eriksson, 1990). Variation in Cd accumulation between different cultivars of spring bread wheat and durum wheat has been observed in a field study in southern Sweden (Stolt, Hultin & Asp, 2002).

The Cd concentration in crops varies considerably with geographical region (Andersson & Pettersson, 1981). Crops grown on alum shales in Norway contain several times more Cd than crops grown on other soils (Singh *et al.*, 1995). In addition to the influence of the parent material, geographical differences may be due to atmospheric deposition, pH, salinity, soil texture, organic matter content or usage patterns of fertilizers and soil amendments (Grant *et al.*, 1999).

Since pH is one of the most important factors influencing the solubility of Cd in soils, it would be reasonable to expect that liming to increase the pH would decrease plant uptake of Cd. However, the effects of liming in field studies have been conflicting; both decreases and increases in crop Cd concentrations as well as no effects have been reported (Andersson & Simán, 1991; Oliver *et al.*, 1996; Jansson & Öborn, 2002). Little or no effects of liming can in some situations be due to a significant Cd uptake from the subsurface soil, which is not affected by liming (Li *et al.*, 1996). Increased Cd uptake with increasing soil pH may in some soils be explained by low affinity for Cd and minimal changes in Cd affinity with pH due to few pH-dependent charges. A small increase in Cd retention by the soil colloid surfaces due to increasing pH would therefore be offset by the increased Ca^{2+} concentrations in soil solution driving Cd^{2+} out from the sorption sites and thus increasing the solution Cd (McLaughlin *et al.*, 1996).

Fertilizers can affect Cd phytoavailability directly, through addition of Cd as a contaminant in P fertilizers, or indirectly through effects on soil conditions and crop growth (Grant *et al.*, 1999). Fertilizers can influence Cd speciation and complexation, which will influence movement of Cd to the root and perhaps also its absorption into the root. Rhizosphere composition, root growth and general crop growth can also be affected by the application of fertilizers. Nitrogen fertilizers can increase Cd concentrations in plants, although the fertilizer does not contain significant levels of Cd. The increased crop uptake of Cd may be caused by ion-exchange reactions with Cd and competing ions from the fertilizer at sorption sites in the soil, or soil acidification (Grant *et al.*, 1999). The type of N fertilizer determines the effect of the fertilization. NH_4^+ fertilizers often cause higher Cd concentrations in crops than NO_3^- fertilizers as a result of the decrease in pH caused by nitrification or plant uptake of NH_4^+. However, in soils of high pH, the effect of the counter ion, *e.g.* Ca^{2+} in $Ca(NO_3)_2$ which is the main component in nitrate of lime, may be greater than the effect of the fertilizer. The counter ion, in this case Ca^{2+}, may substitute for bound Cd, leading to a higher Cd concentration in the soil solution. At high pH values, $Ca(NO_3)_2$ can therefore cause higher Cd concentrations in crops than NH_4^+ fertilizer. In an experiment, the application of N fertilizer to Swiss chard (*Beta vulgaris* L.) growing in a sludge-treated soil increased the Cd uptake (de Villaroel, Chang & Amrhein, 1993). With an increased yield, the Cd uptake increased by 50%, but the Zn uptake was unchanged. The conclusion was that the uptake of Cd and Zn was limited by the rate of desorption of the metals. N fertilization favoured root growth which appeared to facilitate desorption of Cd in the rhizosphere. Jönsson & Eriksson (2003) found in a field study that a higher rate of N fertilizer resulted in higher Cd concentrations in wheat grain. In contrast, other authors have reported decreasing Cd concentrations in grain with increasing N-fertilizer rate. For example, Landberg & Greger (2003) who also studied wheat, but in the laboratory, in both nutrient solution and soil, explained this decrease in Cd concentration by a dilution effect caused by an increase in biomass production. In a long-term experiment Gavi, Basta & Raun (1997) found no effects of N fertilization on the grain content of Cd. Instead, grain Cd was related to soil acidity. Potassium fertilizers can also increase Cd concentrations in crops. However the effect is due more to the anion of the salt, for example Cl^- in KCl has been found to increase the Cd concentration of barley grain. Cl^- ions form soluble complexes with Cd, which results in a decreased sorption of Cd and a greater Cd availability for plants (Grant, Bailey & Therrien, 1996).

Prediction of the Cd concentration in winter wheat grain

In Sweden, cereal-producing farmers who are associated with a programme for safe food certification (Swedish Seal of Quality) have to deliver cereals for human consumption produced with specified methods, with a Cd concentration below a certain level (http://www.svensktsigill.com, 18-Feb-2005). A method for prediction of the Cd concentration in ready-to-harvest winter wheat for a specific field and year would simplify the handling and delivery of winter wheat for human consumption. Eriksson *et al*. (1996) have shown that there is a relationship between Cd concentration in crops and different soil factors such as pH and content of Cd, Zn, organic matter and clay. This has been determined by statistical analyses (multiple regression) for grains of winter wheat, spring wheat and oats, potatoes and carrots. In winter and spring wheat, 30% of the Cd concentration is explained by the soil factors. The corresponding figures are 35% for oats, 64% for carrots and 20% for potatoes. However, these relationships are too weak to be used to predict the Cd uptake at specific sites with any accuracy. Hence, there is a need for a more precise method than prediction from soil analyses. One possibility would be to predict the Cd concentration in grain from plant samples taken at a rather early stage of development. To be able to do this, one has to find a relationship between the Cd concentration in the shoot at an early stage of development and the Cd concentration in harvested grain. Stolt, Hultin & Asp (2002) found in a field study with spring bread wheat and durum wheat that a high Cd concentration in the shoot in the beginning of the growing season also resulted in a high Cd concentration in the grain. These results indicate that it would be possible to predict the Cd concentration in wheat grain from shoot samples at earlier development stages. According to the authors, the results also indicate that grain Cd accumulation is not regulated in the shoot, but in the root or by its Cd uptake.

Objectives

The aims of this thesis were to investigate:

- How and to what extent application of N fertilizer to winter wheat affects the Cd concentration in grain under field conditions and how consistent such effects are between different sites and cultivars
- Whether the Cd concentration in grain of winter wheat is affected by a split N application rate and the time of N application
- When the transport of Cd and N to shoots occurs in winter wheat and how Cd and N are distributed within the shoot at different development stages
- Whether there is a correlation between the Cd concentration at earlier stages in the development of winter wheat and the concentration in harvested grain.

Materials and methods

The study sites

Papers I and II are based on sampling for Cd analysis in existing N fertilization and cultivar trials in winter wheat in 2002 and 2004. In addition, a study of different N fertilization strategies in winter wheat was carried out in 2003 and 2004. Location, temperature and precipitation of all the sites are summarized in Table 1.

Design of field trials and sampling

Trials with different N application rates and cultivars

In 2002 (Papers I and II), samples were collected in the three N-fertilization trials. Bränneberg is situated in the south-west of Sweden, Brunnby in east-central Sweden and Marstad Backa in the south-eastern part (Table 1). The soil at Bränneberg was classified as a Cryaquept, and the soils at Brunnby and Marstad Backa as Eutrocryepts (Soil Survey Staff, 1998). The mean annual air temperature is about 6 ºC at all three sites and the mean annual precipitation 493-607 mm (Table 1). The experimental plots were organized according to a randomized block design with two replicates per treatment combination. Two cultivars of winter wheat (*Triticum aestivum* L., cvs. Kosack and Tarso) and four application doses of the N fertilizer nitrate of lime (75+25, 110+35, 140+50 and 175+60 kg N ha^{-1}) were studied. Nitrate of lime contains 14.4% NO_3^--N and 1.1% NH_4^+-N and most of the nitrate occurs as $Ca(NO_3)_2$. There was also a non-fertilized control plot with Kosack wheat. N fertilizer was applied on two occasions, in late April and late

Table 1. *Location and some selected climate characteristics of the sites*

Site	Trial[1]/ Paper	Latitude	Longitude	Mean annual temp. (ºC)[2]	Mean annual precipitation (mm)[2]
Brunnby	N 02/I,II	59º37’ N	16º40’ E	6.2	607
Bränneberg1	N 02/I,II	58º20’ N	13º04’ E	6.1	558
Marstad B.	N 02/I,II	58º23’ N	15º02’ E	6.0	493
Bränneberg2	N 04/II	58º20’ N	13º04’ E	6.1	558
Fransåker	N 04/II	59º39’ N	17º49’ E	5.8	536
Fasma	C 04/II	60º04’ N	17º38’ E	5.3	606
Fatterslund	C 04/II	55º38’ N	13º09’ E	8.2	569
Russelbacka	C 04/II	58º25’ N	13º01’ E	6.2	602
Svedberga	C 04/II	56º09’ N	12º41’ E	8.2	639
Fiholmsby1	NS 03/T	59º26’ N	16º44’ E	6.4	575
Sättra	NS 03/T	59º33’ N	17º55’ E	5.8	558
Fiholmsby2	NS 04/T	59º26’ N	16º44’ E	6.4	575
Staby	NS 04/T	59º43’ N	17º18’ E	5.5	529

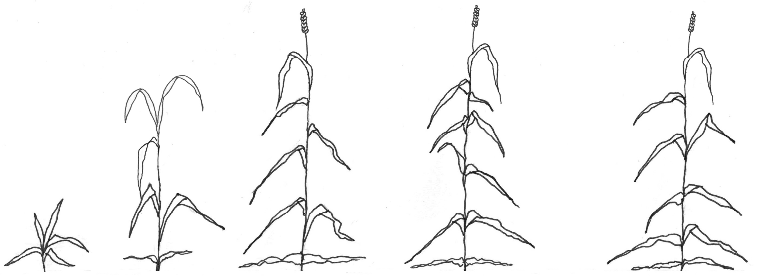
[1] N 02=N fertilization trial 2002, N 04=N fertilization trial 2004, C 04=cultivar trials 2004, NS 03=N fertilization strategy trial 2003, NS 04= N fertilization strategy trial 2004, T=Thesis.

[2] Long-term averages (1961-1990) from nearest official meteorological station.

May/early June, at the later stage of stem elongation. The field trials were treated with herbicides and when necessary with fungicides.

Shoots were sampled on five occasions at intervals of approximately three weeks at the following development stages: (1) stem elongation, (2) ear formation, (3) late flowering - milk ripeness, (4) yellow ripeness and (5) at harvest (Fig. 1). The wheat shoots were cut 5 cm above the soil surface in 2x10-cm-long rows at four randomly chosen spots in each experimental plot. The samples were dried at 30 ºC before the shoots were divided, when applicable, into straw, flag leaf and ear. The grains and husks of the ears of samplings (4) and (5) were separated in a threshing machine. In Paper I, only data for mature wheat grains from the last sampling are presented. All shoot parts except the grains were ground. A small sub-sample was ground for those grain samples that were analyzed for total nitrogen content. Soil samples for site characterization were taken after harvest with augers at the depths 0-25 (0-20 at Bränneberg), 25-50, 50-75 and 75-100 cm. The soil samples were pooled to one composite sample per horizon, air-dried at 35-40 ºC and sieved to pass 2 mm. Data for the horizons deeper than 25 cm are only presented in Paper I.

To study the correlation between shoot Cd concentration in early development stages and Cd concentration in grain, we collected more samples in 2004 to obtain more data (Paper II). Two new fertilization trials of the same series as in 2002 were sampled, one at Bränneberg and the other at Fransåker in east-central Sweden. The N fertilizer in these trials had been changed to ammonium nitrate and



Sampling	1	2	3	4	5
DC	31-37	45-49	69-73[1] / 75-77 [2]	~ DC 87	
Stage of development	stem elongation	ear formation	late flowering-milk ripeness	yellow ripeness	at harvest

[1] Sampling year 2002

[2] Sampling year 2004

Fig. 1. Sampled development stages of winter wheat according to the decimal scale (Zadoks scale) (Zadoks, Chang & Konzak, 1974).

with only a supplementary application of nitrate of lime. Unfortunately, the cultivars Kosack and Tarso were no longer included in these trials. Instead, two similar cultivars, Olivin and Tommi, were sampled. To obtain more data on Kosack and Tarso we also sampled four other trials testing different wheat cultivars. In these trials we sampled Tommi and Olivin as well as Kosack and Tarso. The sites were Fatterslund and Svedberga in the south of Sweden, Fasma in east-central Sweden and Russelbacka in the south-western part. These trials had only one N-fertilizer rate, that recommended for winter wheat in each region, which varied between 153 and 187 kg ha^{-1}. The lower N rates were used in east-central Sweden where yields are lower. At all six sites in 2004, shoots were sampled at three development stages: ear formation, milk ripeness and at harvest (Fig. 1). Soil samples for characterization of the sites were taken after harvest with augers at a depth of 0-25 cm.

Trials with different N fertilization strategies

In order to study whether different N fertilization strategy affected the Cd concentration in winter wheat grain, we used stored grain samples from field trials harvested in 2003 and 2004. We studied samples from four sites in east-central Sweden, two from each year. The field trials had a randomized block design. In 2003 we could only use composite samples (4 replicates) and 3 out of 4 replicates respectively, at the two sites. In 2004 all 4 replicates were available. Sixteen treatments with varying total amounts of N fertilizer, number of applications and times for application were sampled (see Table 3, under Results and Discussion). In 2003 the nitrogen fertilizer used was nitrate of lime. In 2004 the basic nitrogen fertilizer was ammonium nitrate, but late complementary applications were made with either nitrate of lime or ammonium nitrate. At maturity the field trials were harvested and the grains were sampled.

Plant analyses

For Cd determination, 1 g of the ground plant parts and 2 g of whole grain were dissolved by digestion in 10+5 ml concentrated HNO_3 (supra pure) for 20 h at a final temperature of 135 ºC using a Tecator Digester. The Cd concentration was determined by graphite furnace atomic absorption spectrophotometry (GFAAS) on a Perkin-Elmer, Zeeman 4110 ZL in 2002 and on an ICP-MS (Perkin-Elmer, Elan 6100 DRC) in 2004. Reference material (wheat grain and flour) and blanks were included in each digestion and Cd determination. The total nitrogen content of ground plant samples was measured using a Leco CNS-2000 analyzer (Papers I and II). The dry matter (DM) content of the plant samples was determined by oven drying for 16 h at 105 ºC.

Soil analyses

The following soil analyses were carried out for the studies described in Papers I and II. The pH was measured in deionized water (soil-solution ratio 1:5 by weight) (SIS, 1994). For Cd determination, 5 g of soil were extracted in 50 ml 7 M HNO_3 for 2 h at 120 ºC using a Tecator Digester (SIS, 1997), after which the Cd concentration of the extract was measured by GFAAS (Perkin-Elmer, Zeeman 4110 ZL). Total carbon was determined on finely ground soil samples using a Leco CHN-932 elemental analyzer. The exchangeable Cd (Paper II) was determined by a 1-hour shaking with 1 M ammonium nitrate (Symeonides & McRae, 1977) and analysis of the extract on an ICP-MS (Perkin-Elmer, Elan 6100 DRC). The DM content of the soil samples was determined by oven drying for 16 hours at 105 ºC.

Statistical analyses

The results of the plant analyses were treated statistically by regression analysis using Excel (Microsoft Corporation, 2002) for Paper I and Systat (SPSS Inc., 2000) for Paper II. The results of the study of N fertilizing strategies were treated statistically by analysis of variance (the GLM procedure) and Tukey's t-test using SAS (SAS, 2000). In the evaluation of the results from the field trials, $p \leq 0.05$ was considered significant and is used accordingly in the text. Significance levels used in the figures were $p \leq 0.05$ (*), $p \leq 0.01$ (**) and $p \leq 0.001$ (***).

Results and discussion

Soil properties of the study sites

The texture class of the topsoil varied between the sites, from silt loam to clay (Table 2). The pH was moderately acid to slight alkaline (5.5-7.2). The soil Cd concentration extracted by 7M HNO_3 was in the range 0.13 to 0.29 mg kg^{-1} at the sites included in Papers I and II. These values are within the normal range for Swedish agricultural soils (average 0.23 mg kg^{-1}) (Eriksson, Andersson & Andersson, 1997). Extraction by 1M NH_4NO_3 gave Cd concentrations in soil from the same sites that varied between 0.002 and 0.041 mg kg^{-1}. This corresponds to 1-20% of the HNO_3-extractable fraction.

Effect of N fertilizer application on Cd concentration in grain of winter wheat (Paper I)

In this study the effect of different N-fertilizer rates on the Cd accumulation in winter wheat grain was investigated. The grain Cd concentration increased with increasing N rate for the cultivars Kosack (Fig. 2) and Tarso (Fig. 3), although the Cd concentration levels in both soil and grain varied between sites. This effect of N fertilization on Cd concentration agrees with other studies (Singh et al., 1992; Oliver et al., 1993; Grant, Bailey & Therrien, 1996; Mitchell, Grant & Racz, 2000). In contrast to this, Landberg & Greger (2003) found in one pot study and one hydroponic study that the Cd concentration decreased with increasing N rate. However, under field conditions it seems likely that N fertilization generally causes an increase in grain Cd concentration. Landberg & Greger (2003) explained the decreased Cd concentration by a dilution effect caused by an increased biomass production. However, since the increased N rate in our study only gave a small yield increase from the 100 kg ha^{-1} level upwards, no dilution effect of importance could have occurred.

The Cd concentration level in both soil and grain was lower at Bränneberg than at the other two sites, Marstad Backa and Brunnby. The mean Cd concentration in grain at Bränneberg was approximately half that at the other two sites. At Bränneberg and Marstad Backa, the Cd concentration increased gradually with increasing N level, while at Brunnby the increase was more irregular, with a rather large change between two N levels (100 and 145 kg ha^{-1} for Kosack and 145 and 190 kg ha^{-1} for Tarso) and no change between the others. The total increase was stronger at Marstad Backa than at the other sites, which may be due to differences in soil or site properties. An increase of 10 kg N resulted in an increased Cd concentration of 0.001-0.003 mg kg^{-1} at the three sites. An application rate of 145 kg N ha^{-1} is a rather normal N-fertilizer rate for Swedish conditions. If that rate is increased by 30 kg to achieve higher protein content in wheat grain, according to our data the Cd concentration may increase by 6-14%.

Table 2. *Some selected characteristics of the topsoils at the study sites*

Site	Trial[1]/ Paper	Texture class[2]	Org. C %	pH	Cd-HNO_3 mg kg^{-1}	Cd-NH_4NO_3 mg kg^{-1}
Bränneberg1	N 02/I,II	Clay loam	2.7	6.3	0.13	0.005
Brunnby	N 02/I,II	Silt loam	1.3	5.5	0.20	0.041
Marstad B.	N 02/I,II	Clay loam	2.7	6.8	0.29	0.004
Bränneberg2	N 04/II	Clay loam	1.6	6.8	0.13	0.003
Fransåker	N 04/II	Silty clay	2.2	6.5	0.28	0.013
Fatterslund	C 04/II	Clay loam	2.2	7.1	0.26	0.011
Svedberga	C 04/II	Clay loam	3.1	6.9	0.18	0.004
Russelbacka	C 04/II	Silt loam	3.1	7.2	0.15	0.003
Fasma	C04/II	Silty clay	1.7	6.5	0.18	0.002
Fiholmsby1	NS 03/–	Clay	–	6.9	–	–
Sättra	NS 03/–	Clay loam	–	6.1	–	–
Fiholmsby2	NS 04/–	Silty clay	–	6.8	–	–
Staby	NS 04/–	Clay	–	6.4	–	–

[1] N 02=N fertilization trial 2002, N 04=N fertilization trial 2004, C 04=cultivar trials 2004, NS 03=N fertilization strategy trial 2003, NS 04= N fertilization strategy trial 2004.

[2] Classified according to Guidelines for Soil Profile Description (FAO, 1990).

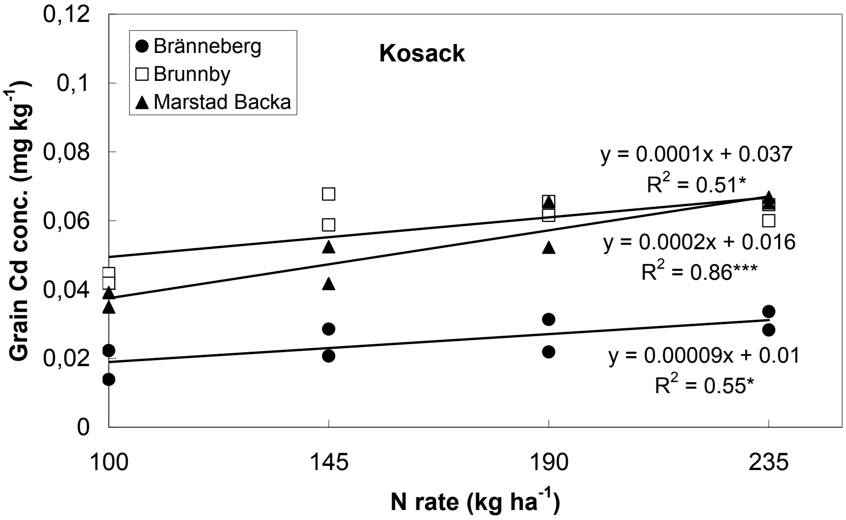


Fig. 2. Grain Cd concentration at different N application rates for cultivar Kosack over three sites (2 replicates).

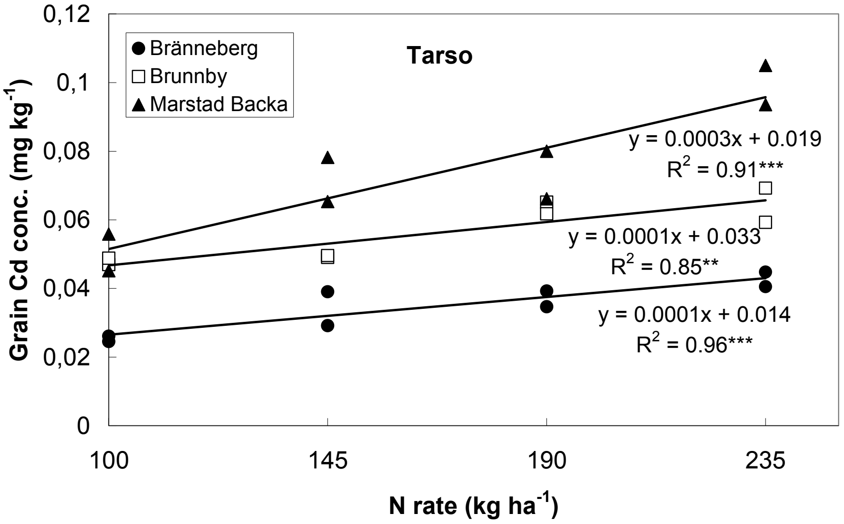


Fig. 3. Grain Cd concentration at different N application rates for cultivar Tarso over three sites (2 replicates).

There were no or only small differences between cultivars in their response to the increased N rate. Tarso had a higher Cd concentration than Kosack at all sites except Brunnby, where the two cultivars had approximately the same concentration. The Cd concentration in wheat grain also increased with increasing N concentration in grain (Fig. 4). The increased Cd uptake may not necessarily be a direct effect of the increased N uptake, which is an effect of the increased N application rate. Instead, the increased N application rate may cause an increased Cd concentration in grain by increasing the concentration of Ca^{2+} in the soil solution. Ca^{2+} is the balancing cation of the fertilizer nitrate of lime. The increased concentration of Ca^{2+} may force Cd^{2+} bound to the soil into the soil solution by an ion-exchange reaction (Brown, Grant & Racz, 1994). There could also be a plant physiological explanation for the increase in grain Cd concentration. Cd^{2+} is bound to sites in root cell walls and this reduces Cd transport into the cell (Rauser, 1987). The increased concentration of Ca^{2+} in the rhizosphere may have reduced the binding of Cd^{2+} in the root cell walls due to ion-exchange reactions. Furthermore, N fertilization reduces root depth, which could have resulted in a higher proportion of the Cd uptake taking place in the topsoil, where the Cd concentration is higher.

Effects of different N application strategies on Cd concentration in winter wheat grain

Since we had previously observed an increase in grain Cd concentration due to N fertilization, we proceeded to investigate whether it is possible to counteract this effect by earlier and/or split N application rates. If the increase in Cd concentration is due to an ion-exchange effect caused by the fertilizer, this effect may be smaller with earlier or split rates. With an early application, some of the Cd released may

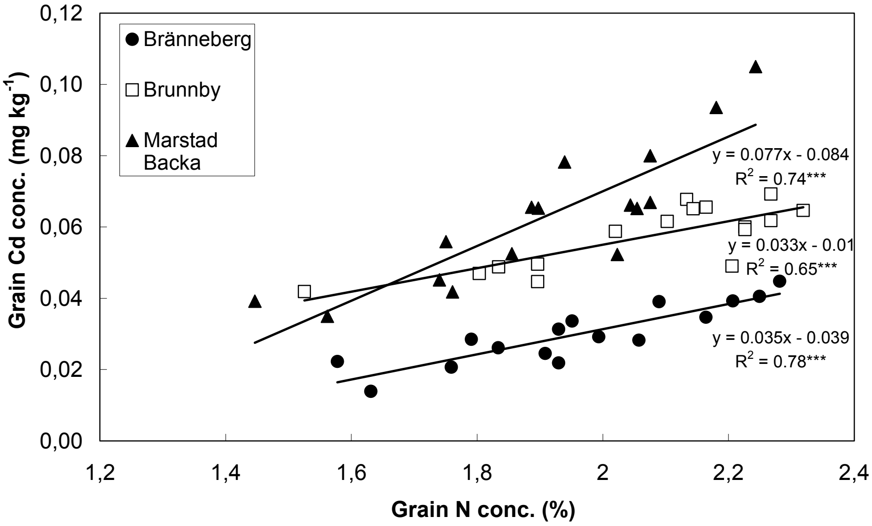


Fig. 4. Correlation between grain Cd concentration and grain N concentration over three sites. Each regression line represents both cultivars, Kosack and Tarso.

be adsorbed again before plant uptake, when leached downwards in the profile. With a split application each individual application is smaller, which could lead to a lower total release of Cd into the soil solution. On the other hand, if the Cd uptake is connected to the N uptake, a more effective N uptake due to a split rate would cause an increased Cd uptake.

The statistical analysis indicated significant differences in grain Cd concentration between N levels at each site, although not all N levels were significantly different from each other. However, there was a general trend that the grain Cd concentration increased with increasing N rate (Table 3), which is in agreement with the results presented in Paper I. Unfortunately, it did not seem as though an earlier or split N application rate could counteract this effect. There were no significant differences between treatments with the same total N rate. Neither a split N application rate nor the time for N application affected the Cd concentration to any significant extent (Table 3). The effects were small and not consistent. The second, complementary, application of N was made with two different fertilizers; ammonium nitrate and nitrate of lime in 2004. According to the statistical analysis there were no significant differences ($p \leq 0.05$) in grain Cd concentration between the fertilizers (Table 3; treatment I versus J, treatment K versus L).

To summarize the study, none of the studied N application strategies was able to reduce the Cd concentration in grain of winter wheat. Neither a split application rate nor an earlier application or change of complementary fertilizer seemed to have any effect.

Table 3. *Grain Cd concentration in the field trials testing different N-fertilizing strategies. The statistical analysis indicated significant differences ($p \leq 0.05$) between N levels but not between treatments on the same N level. The N fertilizers were nitrate of lime in 2003 (at Sättra 03 and Fiholm 03), and ammonium nitrate and nitrate of lime (in italics) in 2004. Early=1/2 April, Normal=27 April, DC 31=early stem elongation, DC 37=late stem elongation, DC 45=ear formation. Number of replicates: Sättra 03=1, Fiholm 03=3, Staby 04=4, Fiholm 04=4*

Treatment	Time of N application					Total N rate	Site			
	Early	Normal	DC 31	DC 37	DC 45		Sättra 03[1]	Fiholm 03	Staby 04	Fiholm 04
	N application rate (kg ha^{-1})						Cd conc. (mg kg^{-1})			
A						0	0.056	0.028	0.029	0.031
B		80				80	0.062	0.048	0.034	0.038
C		120				120	0.063	0.047	0.043	0.041
D		80		*40*		120	0.062	0.057	0.040	0.043
E			120			120	0.069	0.055	0.042	0.045
F		160				160	0.071	0.064	0.041	0.047
G		120		*40*		160	0.071	0.059	0.047	0.047
H	40	80		*40*		160	0.064	0.053	0.048	0.042
I		120			*40*	160	0.073	0.063	0.050	0.051
J		120			40	160	–	–	0.048	0.044
K		80		*80*		160	0.075	0.065	0.043	0.048
L		80		80		160	–	–	0.046	0.050
M		80	80			160	0.072	0.057	0.043	0.044
N		160		*40*		200	0.073	0.068	0.049	0.054
O	40	120		*40*		200	0.070	0.054	0.043	0.048
P		200		*40*		240	0.071	0.078	0.054	0.053

[1] Sättra 03 had no replicates and was therefore not included in the statistical analysis. For each treatment there was a composite sample which consisted of 4 replicates.

Uptake and distribution of Cd and N in winter wheat (Paper II)

The above-ground parts of the plants, *i.e.* the shoots, were sampled at different development stages in order to study how the transport of Cd and N to the shoot changes with time. At each development stage different shoot parts were sampled in order to determine the distribution of Cd and N within the shoots.

The general pattern was that the Cd and N amounts increased in the shoots from the first to the last sampling occasion. Fig. 5 shows an example from the site Marstad Backa. An exception to the general pattern was the last sampling at Bränneberg, which is further discussed in Paper II. During the study period the Cd content in the shoots increased by a factor of approximately 4 and the N content approximately doubled at the three sites investigated. The flag leaves were sampled separately in order to see whether redistribution of Cd from the flag leaf to the grain had an influence on the final grain Cd concentration at harvest. However, the biomass of the flag leaves constituted only a small proportion of the total shoot biomass and the Cd concentration was at the same level. Thus, the Cd content in the flag leaves was of minor importance for the Cd concentration in grain at harvest.

Nitrogen seemed to be redistributed from the vegetative parts to the ear as soon as it emerged. The pattern was different and more irregular for Cd. The Cd content in the vegetative parts increased until harvest at Marstad Backa and sampling 4 at Brunnby. This indicates that Cd is redistributed to the grain to a much lesser extent than N and that any such redistribution takes place at a later stage of development. There could still have been redistribution of Cd when the Cd content in the vegetative parts increased, but the redistribution should then have been compensated for by an uptake from the soil or from the roots. The results make it difficult to draw

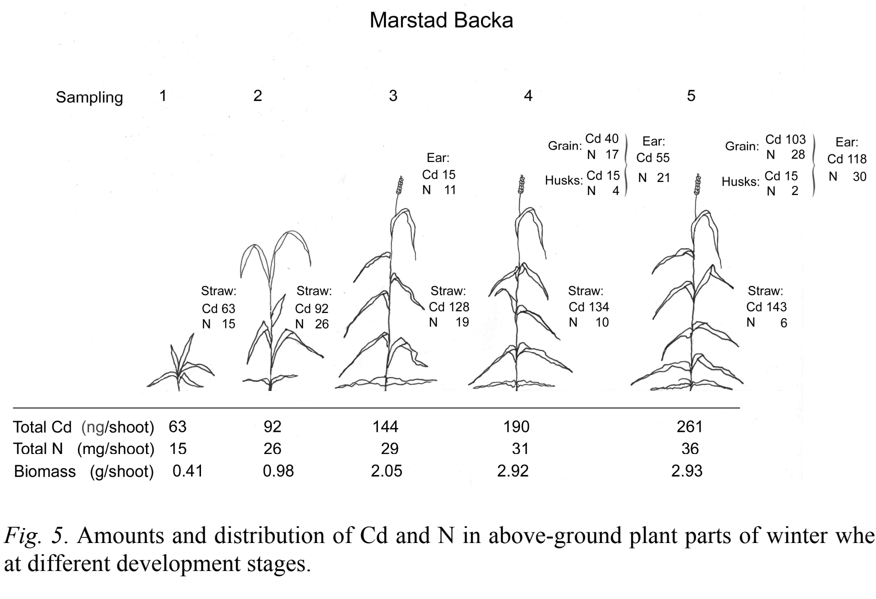


Fig. 5. Amounts and distribution of Cd and N in above-ground plant parts of winter wheat at different development stages.

any definite conclusions concerning the redistribution of Cd. Furthermore, the results do not give any clear evidence for a link between the transport of Cd and N in the shoot. Such a link could have explained why an increased N rate gives an increased Cd concentration in grain, as was found in Paper I.

Grain Cd concentration as a function of early season shoot concentration (Paper II)

Shoot samples from earlier development stages and mature grain samples from the same experimental plots were analyzed for Cd in order to determine whether there was a relationship between the Cd concentrations in shoots at the earlier development stages and in mature grain. In the first year of sampling (2002), the relationship was tested at stem elongation, ear formation and milk ripeness (Fig. 6). There was a relationship between the Cd concentration in grain at harvest and the Cd concentration in shoots already at stem elongation (Fig. 6a). However, the cultivar Tarso at Marstad Backa deviated from this pattern, and showed another, but rather distinct, relationship between shoot and grain Cd concentration. There was also a correlation between shoot and grain Cd concentration at ear formation and milk ripeness (Figs. 6b and c). Tarso at Marstad Backa again deviated from the general pattern. The correlation between shoot and grain Cd concentration was strongest at ear formation. In 2004, the correlation was only tested at ear formation and milk ripeness. The correlations found in this year were similar to those in 2002. Again, one cultivar at one site deviated from the general pattern, namely Olivin at Fransåker. The rather consistent pattern between the two years makes it possible to merge both years into one figure and still get a good correlation at ear formation (Fig. 7). Apart from the discrepancies at Marstad Backa and Fransåker, there seems to be a common relationship for data from different sites and cultivars.

There were also significant relationships between the shoot and grain Cd concentration for the individual cultivars. Fig. 8a shows that the relationships for Tarso and Kosack over the two sampling years were similar to each other at ear formation. The data for Tarso at Marstad Backa, which deviated from the general pattern, were not included in the regression. The cultivars Olivin and Tommi also showed significant relationships between shoot and grain Cd concentrations (Fig. 8b). The Fransåker site was regarded as apart and excluded from the regressions. Olivin had a regression line similar to those of Kosack and Tarso (*cf.* Figs. 8a and b), while Tommi deviated from the general pattern. However, when Fransåker (filled symbols) was included in the regression analysis Olivin, and not Tommi, then deviated from the general pattern. For further discussion see Paper I. In any case, the results indicate that either Olivin or Tommi does not conform to the general relationship between shoot and grain Cd concentration among the cultivars investigated.

For most of the sampled material there was a correlation between shoot and grain Cd concentration that was consistent between years, sites and cultivars. However, there were deviations from the general pattern that are difficult to ex-

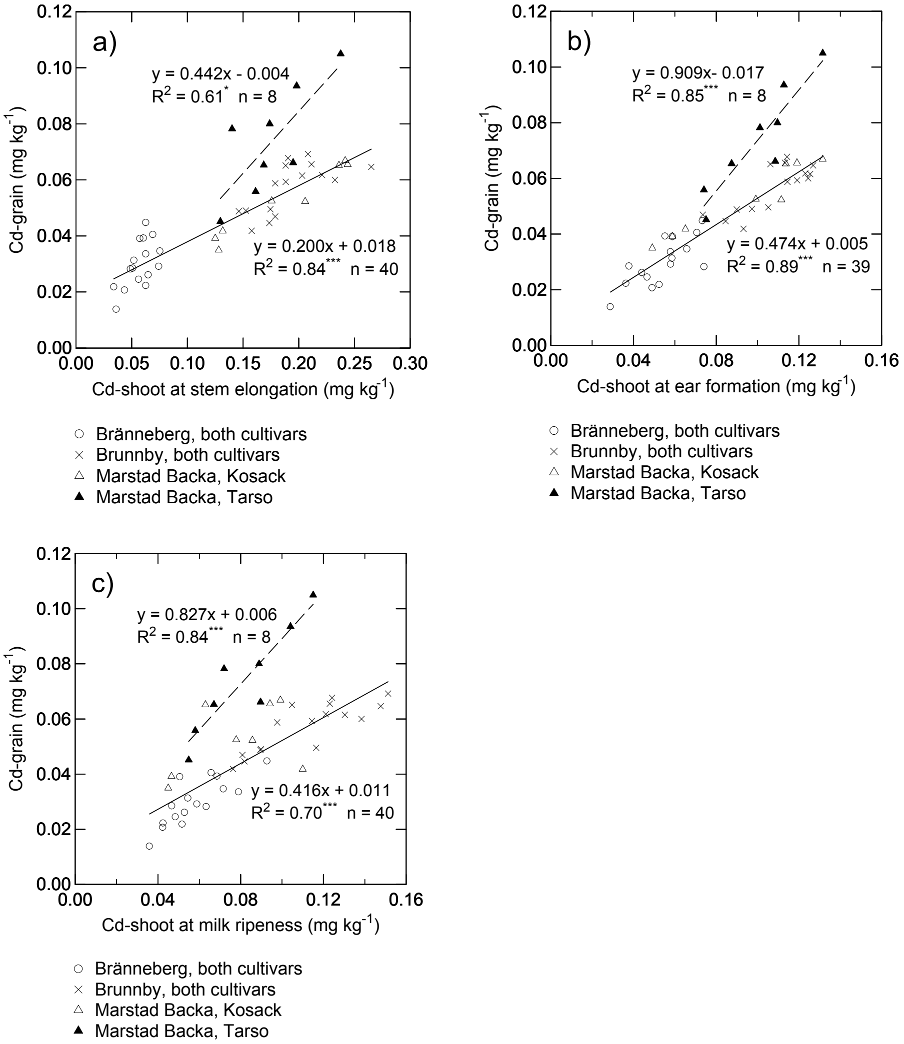


Fig. 6. Cadmium concentrations in grain at harvest as a function of shoot Cd concentrations at a) stem elongation, b) ear formation and c) milk ripeness in 2002. Filled symbols belong to the hatched regression line (Tarso at Marstad Backa).

plain. Unfortunately, these deviations, at Fransåker and in Tarso at Marstad Backa, were characterized by relatively high Cd concentrations. Thus, these high grain Cd values would have been underestimated if we had used a regression line common for all sites. This is a problem since it is important to predict these high values. However, in most cases the deviating samples had their own significant relationships. Another reason why a few samples deviated from the regression line in Fig. 8b could be that the relationships were not rectilinear. Still, in most cases the deviating samples had their own rectilinear relationships between shoot and grain Cd concentration. Therefore it seems likely that the relationships are rectilinear. By making more investigations it might be possible to develop specific correlations for different cultivars, regions or soils.

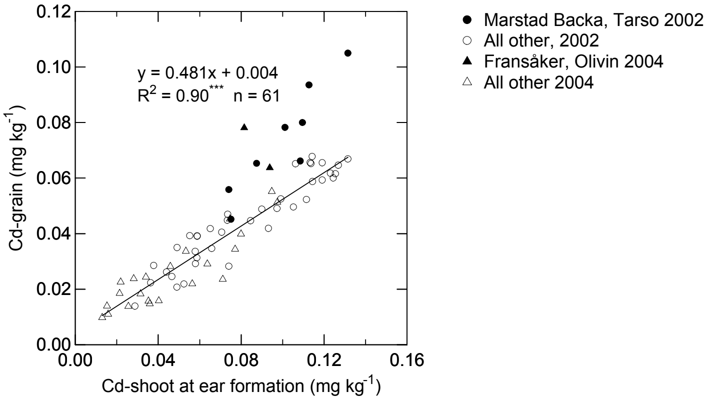


Fig. 7. Cadmium concentrations in grain at harvest as a function of shoot Cd concentrations at ear formation. Data from 2002 and 2004 merged. Filled symbols were not included in the regression analysis.

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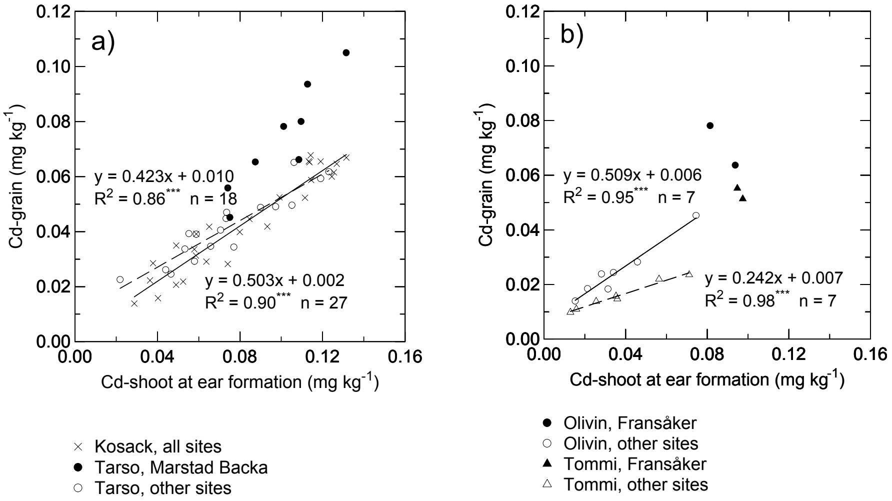

Fig. 8. Cadmium concentrations in grain at harvest as a function of shoot Cd concentrations at ear formation for a) cultivars Kosack and Tarso in 2002 and 2004, and b) cultivars Olivin and Tommi in 2004. Filled symbols were not included in the regression analysis.

Concluding discussion

As shown by the results presented in this thesis, increasing the application rate of N fertilizer from 100 kg N ha^{-1} to 235 kg N ha^{-1} increased the Cd concentration in mature grains of winter wheat under field conditions (Paper I). The increase occurred independently of site and cultivar. Increasing the N application rate from 145 to 175 kg N ha^{-1}, in order to increase the protein content of bread wheat, would increase the grain Cd concentration by 6-14%. At sites where the Cd level is already high, such an increase might result in a Cd concentration that exceeds the applicable limit value for wheat grain. The EC limit value for wheat grain is 200 µg kg^{-1} (EC, 2001), whereas the Swedish Seal of Quality has a limit value of 80 µg kg^{-1} (Swedish Seal of Quality, 2005). We also found a correlation between N and Cd concentrations in wheat grain (Paper I). This is in accordance with data from similar field studies on winter wheat and spring wheat presented by Jönsson & Eriksson (2003).

An explanation for the increase could either be found in the soil or in the plant, but it could also be a combination of the two. Hart *et al.* (1998) found that Cd accumulation in durum wheat may be related to phloem-mediated Cd transport to the grain. In our study on winter wheat, both Cd and N increased in the shoot during the entire period investigated. However, the results do not clearly indicate that the two elements are linked to each other in the translocation to and within the shoot (Paper II). Another possible explanation for the increased Cd concentration is that the N fertilizer (nitrate of lime) causes an ion-exchange effect (Paper I). The balancing cation of the fertilizer (Ca^{2+}) might have resulted in an increased Cd concentration in the soil solution and ultimately in the shoots.

We investigated whether different N strategies could reduce the effect of N fertilizer on Cd concentration and found no significant effects. Neither a split N fertilizer rate nor an earlier first application decreased the Cd concentration in grain of winter wheat. However, there are several other ways to decrease the Cd concentration in bread wheat. Since the Cd concentration increases with increasing N fertilizer rate, it is important to keep the rate as low as possible. However, to get a good yield and high protein content in bread wheat, a certain amount of N fertilizer must often be used. Even if a split N application rate does not seem to have a direct effect, it could indirectly reduce the grain Cd concentration, since a split application is easier to adjust to the needs of the crop and thus the risk of excessive application of N fertilizer is reduced. Other methods to decrease the Cd concentration may be cultivar selection, site selection and liming (see also Introduction). There are differences in Cd accumulation between cultivars of the same species. Through plant breeding it may be possible to reduce the Cd concentration in bread wheat in the long-term. On soils with low pH and high Cd concentration, liming might be a method to reduce the Cd concentration in wheat.

The Cd concentration in crops has been shown to vary greatly between regions and sites. Often the largest difference in crop Cd concentration occurs between sites and not between cultivars or different soil treatments. Hence, selection of sites with low Cd concentrations might be an effective means to reduce grain Cd concentration. However, it is not only the Cd concentration that is important for the site selection, since for example the fertility of a site is also an important factor.

Even if efforts have been made to reduce the Cd concentration in wheat grain it may still in some cases be difficult to avoid too high concentrations. Then it would be useful to have a method to predict the grain Cd concentration in due time before harvest. Prediction of the grain Cd concentration by soil analysis does not seem to be sufficiently reliable (Eriksson *et al*., 1996; McLaughlin *et al*., 1999). Our results indicate that early prediction by plant analysis could be possible (Paper II). There were correlations between the Cd concentration in shoots and the Cd concentration in grain at all three development stages studied, starting at stem elongation, i.e. about 2.5 months before harvest. This is in accordance with findings by Stolt, Hultin & Asp (2002); a high Cd concentration in the shoot of spring bread wheat and durum wheat in the beginning of the growing season resulted in a high Cd concentration in grain. The best correlations in our study were obtained at ear formation, about 2 months before harvest, which should be early enough. Some sites/cultivars deviated from the general pattern, but they showed their own rather distinct relationships between shoot and grain Cd concentration. To summarize, our results suggest that after further investigation, Cd prediction by plant analysis may be used to identify fields which are likely to exceed a certain limit value for Cd concentration in wheat grain.

Conclusions

N fertilizer application increased the Cd concentration in grain of winter wheat. The effect was the same at different sites and for different cultivars. If the N-fertilizer rate of 145 kg N ha $^{-1}$, which is a rather normal rate for Swedish winter wheat, is increased by 30 kg to achieve higher protein content in wheat grain, the Cd concentration may increase by 6-14%.

Earlier application of N and split application of N had no effects on the Cd concentration in wheat grain. None of these methods seemed to counteract the effect of an increased N application rate.

The amounts of Cd and N in wheat shoots increased during the entire study period, from stem elongation to maturity. Nitrogen was redistributed to the ear/grain as soon as the ear had developed. For Cd, there was only an indication of a small redistribution at one of the sites taking place just before maturity. The transport of Cd and N did not appear to be linked to each other.

There seemed to be a correlation between Cd concentration in shoots and Cd concentration in grain already at stem elongation. The correlation was strongest at ear formation. However, because of deviations of some cultivars and sites from the general pattern, it is too early to make reliable predictions of the Cd concentration in harvested grain based only on our results.

Suggestions for future research

There is a need for more research on why N fertilization increases the Cd concentration in wheat grain. By studying ion exchange reactions caused by the fertilization one might come closer to the solution. It could also be useful to study how Cd is transported in the plant and how this uptake is affected by the N uptake.

According to our research, it appears as though it may be possible to predict the Cd concentration in wheat grain by analysing the Cd concentration in shoots early in the season. Before such Cd prediction can be useful in practice, further studies that include more soils, cultivars and years are needed.

One possibility to better understand the Cd uptake would be to study the rhizosphere, the part of the soil which is closest to the roots. Eriksson (1990) found that wheat has an ability to take up soil Cd, although strongly bound, while oats does not have that ability to the same extent. By studying easily soluble Cd, pH, electrical conductivity and low molecular acids in the rhizosphere, one might get a better understanding of what is happening in the root zone.

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