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Effect of nitrogen fertiliser applications on cadmium concentrations in durum wheat (*Triticum turgidum*) grain

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Abstract The effect of different rates of nitrogen (N) fertiliser application on cadmium (Cd) concentrations in the grain of two durum wheat (*Triticum turgidum* L.) cultivars was investigated at three different field trial sites in Canterbury, New Zealand. The mean Cd concentration in this study was 66 µg kg^{-1} fresh weight (FW), which is 30% below the maximum level (ML). Uptake of Cd was higher in 'CRDW17' compared with 'Waitohi' at all three sites. Also increasing amounts of calcium ammonium nitrate (CAN) fertiliser increased the Cd concentration in durum wheat grain at all three sites. Wheat grain Cd concentrations were not found to be related to any measured soil properties. Grain Cd concentrations were in most instances positively correlated with total biological yield (TBY). This response may be the result of the combined effects of fertiliser salts on ionic composition of the soil solution, and increased root interception and enhanced mass flow of Cd related to increased TBYs. The evidence suggests that it is TBY rather than grain yield that is more important with regards to Cd uptake in durum wheat.

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INTRODUCTION

The main source of cadmium (Cd) in agricultural soils in New Zealand is from phosphate fertilisers. The long-term application of superphosphate fertiliser has resulted in a significant increase in total soil Cd concentrations (Roberts et al. 1994; Gray et al. 1999a). Once Cd is in the soil, it may accumulate in plants through water uptake in roots (Hart et al. 1998) and then enter the human food chain.

With the exception of smoking, food is the main route by which Cd enters the human body. The Joint Food and Agriculture Organisation (FAO)/World Health Organisation (WHO) Expert Committee on Food Additives (JECFA) recommend a provisional tolerable weekly intake of Cd for an adult (60 kg body weight) to be 420 µg $week^{-1}$ (JECFA 1989). The Australia New Zealand Food Authority (ANZFA), have recently adopted new joint food standard codes for New Zealand and Australia (ANZFA 1997). The new food standards mean that the maximum level (ML) for Cd in grain has fallen from 1000 to 100 µg kg^{-1} fresh weight (FW).

A recent investigation of Cd concentrations in the grain of 44 different New Zealand wheat cultivars grown at 25 different sites showed that the overall mean Cd concentration (i.e., 54 µg kg^{-1} FW) was significantly below the newly adopted ML of 100 µg kg^{-1} FW (Gray et al. 2001). It was also found that sometimes there were large differences in Cd concentrations between wheat grain of the same cultivars grown at different sites. Interestingly however, there were no significant relationships between Cd in wheat grain and major soil factors, such as pH, total soil zinc (Zn), cation exchange capacity (CEC), total Cd, and organic matter which have been shown to influence plant uptake of Cd (McLaughlin et al. 1996; Gray et al. 1999b). One suggestion for the lack of relationships was that crop management practices employed at the different sites, for example fertiliser application, might influence Cd uptake.

The application of nitrogen (N) fertiliser to wheat crops to maximise yield and protein content is a routine management technique used by growers. However, the form of N used may influence plant uptake of Cd. Previous studies have shown that acidifying N fertilisers such as ammonium sulphate can increase Cd uptake in plants compared with alkaline fertilisers (Eriksson 1990; Willaert & Verloo 1992). For example, Williams & David (1976) showed that wheat crops accumulated more Cd when fertilised with ammonium nitrate (NH_4NO_3) in conjunction with superphosphate compared with superphosphate alone. To minimise the acidification of soils, calcium ammonium nitrate (CAN) is frequently used to provide N to wheat crops in New Zealand. To date the effects of this acid-neutral form of N fertiliser on Cd uptake in wheat have not been investigated. Thus, the objective of this study was to investigate the effect of different application rates of CAN on Cd concentrations in the grain of two durum wheat (*Triticum turgidum* L.) cultivars grown at three sites that had a wide range of paddock history and yield potential.

MATERIALS AND METHODS

Experimental sites

Three experiments were established in Canterbury to investigate the effect of N fertiliser on yield and quality of durum wheat. Full details of the experimental sites, the agronomic and grain quality measurements, are available in Reddecliffe (2002). The main aspects relevant to the current study outlined as follows.

Specifically there was a dryland site at Darfield, and irrigated sites at both Wakanui and Lincoln. At each site an experiment was sown as a randomised complete block design with four replicates of 12 treatments comprising two durum wheat cultivars, i.e., 'CRDW17' and 'Waitohi', and six N application rates. The amount of N applied differed among sites but followed the same basic pattern. N was applied as CAN at two rates during early tillering (GS23), and at three rates during flag leaf emergence (GS39) (Table 1). In addition, at the Wakanui site, 50 kg N ha^{-1} was applied to the whole site at sowing. The treatments were designed to provide a range of potential grain yields. The plots were at least 12 m long and 1.35 m wide, and sown at 0.15-m row widths to achieve a target population of 250 plants m^{-2}. At all sites, the farmers used normal agronomic practices to control weeds and diseases and there was no evidence of fungal damage to any crop.

Soil analysis

Before sowing, composite soil samples (10–15 cores, 25 mm diam.) were taken to a depth of 150 mm at each site. Subsamples of soil were air-dried and ground to pass through a 2-mm stainless steel sieve before laboratory analysis. Soil pH was measured in a water suspension using a soil:solution ratio of 1:2.5 (Blakemore et al. 1987). Soil texture was determined by the Malvern Laser Sizer method described in Singer et al. (1988). Total carbon (C) and N content in the soils was determined by LECO CNS 2000 analyser. The CEC was determined by ammonium acetate leaching at pH 7.0 (Blakemore et al. 1987). Bioavailable phosphorus (P) (Olsen P) was determined by bicarbonate extraction. Total Cd and Zn were determined by nitric acid microwave digestion method USEPA SW 846-Method 3051 (USEPA 1990). Cd was measured in the digest by graphite furnace atomic absorption

Table 1 Nitrogen (N) treatments (kg ha^{-1}) applied to 'Waitohi' and 'CRDW17' durum wheat (*Triticum turgidum*) cultivars at three different sites. (GS23 stage = tillering; GS39 stage = flag leaf emergence.)

		N application (kg ha^{-1})	
Site	Treatment	GS23	GS39
Darfield	1	0	0
	2	0	25
	3	0	50
	4	150	0
	5	150	25
	6	150	50
Wakanui	1	0	0
	2	0	50
	3	0	100
	4	150	0
	5	150	50
	6	150	100
Lincoln	1	0	0
	2	0	40
	3	0	80
	4	175	0
	5	175	40
	6	175	80

spectrophotometry (GFAAS) with deuterium arc background correction using 0.1% H_3PO_4 as a matrix modifier. Zn was determined by flame atomic absorption spectrophotometry (FAAS). Extractable Cd was determined by 0.05*M* calcium nitrate ($Ca(NO_3)_2$ 1:10 soil:solution ratio, 16 h extraction). Extracts were centrifuged at 10 000 rpm for 10 min and filtered through a Whatman No. 42 filter paper before analysis of Cd by GFAAS, again using 0.1% H_3PO_4 as a matrix modifier.

Crop yield

The total biological yield (TBY) of each crop was estimated from the total above ground dry matter (DM) measured from two 0.1 m^2 quadrats cut to ground level at physiological maturity. From these samples the crop harvest index (HI) was calculated as: (grain yield/TBY) × 100. The remainder of the crop was header-harvested once grain had dried further and reached harvest maturity (14% moisture content). The grain Cd concentration was measured in these header-harvested grain samples. Unfortunately the grain samples from Lincoln were bulked after measurements of grain yield, but before Cd determination. Therefore, four replicates were used for HI, TBY, and grain yield analysis, and the mean of the four replicates correlated with the single result for grain Cd concentration.

Grain analysis

Samples of c. 100 g wheat grain were finely ground in a Tema Mill and stored in polypropylene containers. Subsamples of ground grain (0.5 g) were digested in 10 ml of 69% Aristar grade nitric acid and heated on a digestion block up to 140°C over a period of 7 h. The digest was then made up to 20 ml with deionised water and filtered through a Whatman No. 42 filter paper. Analysis of all the grain samples was made in triplicate together with reagent blanks. Cd was determined in the digests by GFAAS with deuterium arc background correction and 0.1% H_3PO_4 acid was used as a modifier. The limit of detection (LOD) was 10 μg kg^{-1} dry weight (DW).

Quality control

The reproducibility of the analytical procedure for grain analysis was checked by including four blanks in every batch of 80 samples and two standard samples of durum wheat flour (National Institute of Standards and Technology 8436) in every second batch. The expected mean Cd concentration ± standard error (SE) for this standard sample was 110 ± 50 μg kg^{-1} FW and the mean value obtained was 110 ± 10 μg kg^{-1} FW.

Statistical analysis

Analysis of variance (ANOVA) was conducted using Minitab version 9.2 for the randomised complete block design at each location. Effects were considered significant if they differed at the probability level of 5% based on Fisher's protected least significant difference tests. As a result of only having grain samples from one of the replicates from Lincoln, no statistical interpretation could be made for Cd in grain from this site.

RESULTS AND DISCUSSION

Soils

Total soil Cd concentrations ranged from 0.11 to 0.15 mg kg^{-1} (Table 2). These values lie within the range for total Cd in arable soils in New Zealand as previously reported by Roberts et al. (1995). Calcium nitrate extractable Cd for all three soils was generally low compared with soils in other investigations (Gray et al. 1999c). Other soil properties, which have previously been shown to influence wheat grain and indeed plant uptake of Cd in general also varied, such as soil pH, total C content, clay content, CEC, and total Zn (Table 2). In the present study however, since there were only three sites, it was hardly surprising that there were no significant relationships between grain Cd concentrations and any of these soil properties.

Table 2 Selected soil properties from the three trial sites (0–150 mm soil depth). (CEC, cation exchange capacity.)

Soil property	Darfield	Wakanui	Lincoln
pH	5.5	5.6	5.8
Total C (%)	2.9	2.4	2.7
Total N (%)	0.27	0.23	0.22
CEC (cmol$_c$ kg^{-1})	13.1	13.0	11.9
Olsen P (μg ml^{-1})	16	15	16
Sand (%)	37	18	38
Silt (%)	45	60	51
Clay (%)	18	22	11
Extractable Cd (μg kg^{-1})*	13.8	12.9	8.3
Total Cd (mg kg^{-1})	0.151	0.136	0.113
Total Zn (mg kg^{-1})	49.8	54.3	38.8

*Calcium nitrate-extractable Cd.

Grain yield and total biological yield

The grain yield and TBY differed between cultivars, the timing of N applications, and experimental sites (Table 3). At Darfield, grain yield increased significantly from 3.2 to 3.9 t ha^{-1} for the control treatments up to 5.5 t ha^{-1} for both cultivars at the highest N treatments (Table 3). There were no significant differences in grain yield between the two cultivars. TBY at Darfield also increased with N application, from c. 9 t ha^{-1} in the control plots to c. 15 t ha^{-1} for the highest N treatments. The application of 150 kg N ha^{-1} at tillering resulted in a higher TBY compared with the no-early N treatments. In contrast, at Wakanui there was no significant increase in grain yield for either cultivar with increasing N treatments (Table 3) but 'Waitohi' produced 0.8 t ha^{-1} more ($P < 0.05$) grain than 'CRDW17'. In addition the TBY for both cultivars

Table 3 Mean values of grain yield and total biological yield (TBY) and crop harvest index (HI) for 'CRDW17' and 'Waitohi' durum wheat (*Triticum turgidum*) at three sites with different nitrogen (N) treatments.

Site	Cultivar	Early N* (kg ha^{-1})	Late N† (kg ha^{-1})	Grain yield (t ha^{-1})	TBY	HI
Darfield	CRDW17	0	0	3.2	9.0	0.36
		0	25	4.1	10.8	0.38
		0	50	4.6	10.9	0.42
		150	0	5.5	13.3	0.41
		150	25	5.6	15.3	0.37
		150	50	5.5	15.0	0.37
	Waitohi	0	0	3.9	8.9	0.44
		0	25	4.6	10.1	0.46
		0	50	4.0	10.9	0.37
		150	0	5.6	13.3	0.42
		150	25	5.5	14.7	0.37
		150	50	5.5	14.1	0.39
	LSD (0.05)			1.22	1.97	0.068
Wakanui	CRDW17	0	0	7.2	16.0	0.45
		0	50	7.8	17.9	0.44
		0	100	7.4	18.9	0.39
		150	0	7.0	17.0	0.41
		150	50	6.8	16.5	0.41
		150	100	6.8	19.1	0.36
	Waitohi	0	0	7.6	15.5	0.49
		0	50	7.8	16.3	0.48
		0	100	8.0	19.7	0.41
		150	0	8.0	21.0	0.38
		150	50	8.6	19.8	0.43
		150	100	8.0	20.0	0.40
	LSD (0.05)			0.62	2.73	0.076
Lincoln	CRDW17	0	0	4.5	11.0	0.41
		0	40	4.8	11.6	0.41
		0	80	6.1	13.8	0.44
		175	0	7.3	16.3	0.45
		175	40	7.5	16.0	0.47
		175	80	7.6	17.0	0.45
	Waitohi	0	0	4.5	10.8	0.42
		0	40	5.2	9.2	0.57
		0	80	5.8	12.2	0.48
		175	0	7.8	14.3	0.55
		175	40	8.0	15.0	0.53
		175	80	7.8	15.2	0.51
	LSD (0.05)			0.84	2.24	0.097

*Applied at GS23.
†Applied at GS39.

increased ($P < 0.05$) from the control to the highest N treatment. The application of 150 kg N ha^{-1} at tillering did not increase grain yield or TBY compared with the no-early N treatments. This may have been a result of the basal application of 50 kg N ha^{-1} applied at Wakanui before establishment of the trial. As a consequence it was possible there was no N deficiency and hence no subsequent yield response. At Lincoln, as at Darfield, there were significant increases in grain yield and TBY with increasing rate of N application for both cultivars.

Cadmium concentration in grain

Cadmium concentrations in wheat grain ranged between 28.6 and 99.7 µg kg^{-1} FW with a mean concentration of 65.8 µg kg^{-1} FW (Fig. 1). The mean concentration was higher than that previously found for Cd in grain in New Zealand by Gray et al. (2001) but lower than that found by Roberts et al. (1995). Nonetheless, the mean concentration was still 30% lower than the ML of 100 µg kg^{-1} FW.

Cadmium concentrations were higher in ‘CRDW17’ than ‘Waitohi’ for all six treatments at all three sites (Fig. 1A,B,C). A number of overseas investigations have found a large variation in grain Cd concentrations between different wheat cultivars. For example, Oliver et al. (1995) found considerable variation in grain Cd concentrations in Australian wheat cultivars, and identified trends in cultivar accumulation. Cieslinski et al. (1996) also found Cd accumulation in the grain of durum wheat was strongly affected by cultivar type in a Canadian study. In New Zealand, Gray et al. (2001) found there was a 4-fold range in the abilities of a large number of both winter and spring wheat cultivars to accumulate Cd across a number of different sites.

Comparison between sites

A comparison of Cd uptake between sites was made by comparing the average grain Cd concentration of each cultivar across all treatments at the trial sites. Results showed that for both wheat cultivars, grain Cd concentrations followed the order Wakanui > Lincoln > Darfield. These results were inconsistent with the soil properties, which indicate that Darfield had the highest total and extractable Cd and slightly lower soil pH than the other two sites (Table 2). This suggests that soil pH and Cd concentrations were not the main factors influencing grain Cd concentrations.

A possible explanation for the higher Cd uptake in grain at sites Wakanui and Lincoln compared with Darfield may be irrigation. Darfield was not irrigated during the course of the experiment while Wakanui and Lincoln had irrigation supplied to ensure crops were not moisture stressed. The two irrigated sites (i.e., Wakanui and Lincoln) produced higher yields compared with the non-irrigated site (i.e., Darfield) (Table 3). This may lead to greater Cd uptake into wheat through increased root interception and enhanced mass transport of Cd, driven by higher transpiration in the higher yielding crops.

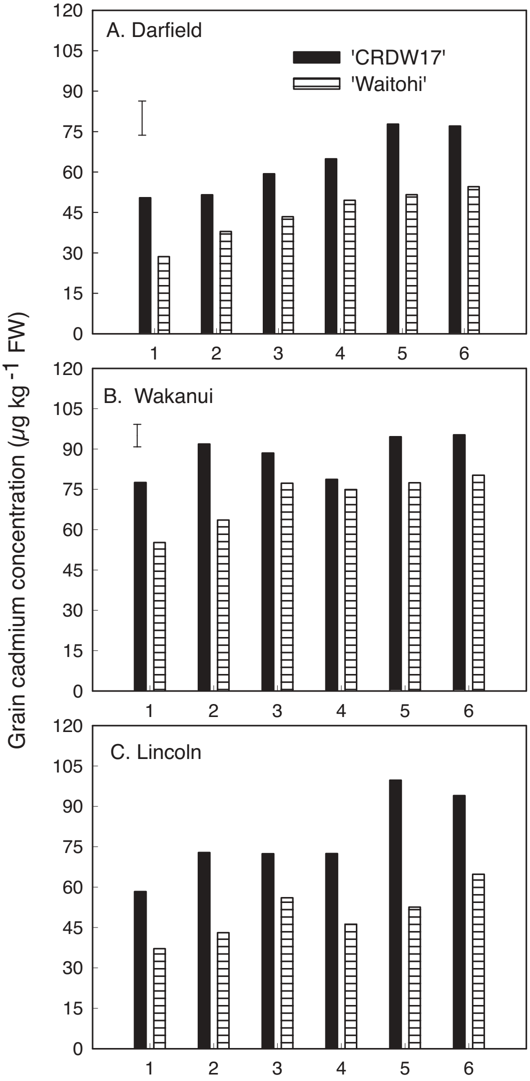

Fig. 1 Grain cadmium (Cd) concentrations (µg kg^{-1}) in ‘CRDW17’ and ‘Waitohi’ durum wheat (*Triticum turgidum*) cultivars with increasing nitrogen application rates at: **A**, Darfield; **B**, Wakanui; and **C**, Lincoln trial sites. Error bar is LSD ($P < 0.05$).

Effect of nitrogen application on cadmium in grain

Cadmium concentrations in the grain of both durum wheat cultivars increased significantly with N fertiliser application when N was applied at both tillering and flag leaf emergence, i.e., Treatments 5 and 6 (Fig. 1A,B,C). This pattern was consistent at all three sites and for both wheat cultivars. When N was applied only at tillering or flag leaf emergence, however, the pattern of Cd concentration in grain was unclear. For example, at Wakanui a single application of 150 kg ha^{-1} N at tillering (Treatment 4) did not significantly increase Cd concentration in 'CRDW17' compared with the control (Treatment 1), whereas an additional application of 50 or 100 kg N ha^{-1} at flag leaf emergence (Treatments 5 and 6) resulted in a significant increase in Cd concentration in this cultivar. At Darfield, an application of 25 or 50 kg N ha^{-1} at flag leaf emergence (Treatments 2 and 3) did not lead to a significant increase in Cd in grain in 'CRDW17'.

There were also differences in the response of cultivars to N application. As described above, at Wakanui an application of 150 kg ha^{-1} N at tillering (Treatment 4) did not significantly increase Cd concentration in 'CRDW17', whereas there was a significant increase in Cd concentration in the grain of 'Waitohi' compared with the control. Similarly for Darfield, an application of 50 kg N ha^{-1} at flag leaf emergence (Treatment 3) significantly increased Cd concentration in Waitohi but not in 'CRDW17' compared with the control.

A number of investigations have shown that increasing applications of N fertiliser to soil can result in increased Cd uptake in crops. Oliver et al. (1993) found in a field trial that the mean Cd concentration in wheat grain increased with increasing rates of N of up to 80 kg ha^{-1} irrespective of the crop rotation. Grant et al. (1996) showed that the application of NH_4NO_3 increased Cd in malting barley when soil nitrate levels were low. In a growth chamber experiment, Mitchell et al. (2000) showed that increased rates of N application as urea increased the concentration of Cd in durum wheat straw and grain.

Mechanisms for enhanced cadmium uptake with nitrogen application

There have been a number of suggestions offered as to why Cd concentrations in grain increase with increasing amounts of N fertiliser. Some N fertilisers, such as NH_4NO_3, acidify the soil over time (Marschner & Romheld 1983; Bolan et al. 1991). This decrease in pH may decrease the number of available sorption sites for Cd on soil colloids and increase the activity of Cd in soil solution. It is soil solution Cd that is considered most readily available for plant uptake. Oliver et al. (1993) suggested this mechanism as the reason for increased Cd uptake in wheat grain after N fertilisation in their field trial.

The application of fertiliser salts may also increase Cd solubility in soils by increasing the ionic strength of the soil solution and displacing non-specifically bound Cd (Naidu et al. 1994). For example, Lorenz et al. (1994) showed in a pot trial experiment that soil solution Cd concentrations were related to excess application of potassium nitrate (KNO_3) and NH_4NO_3 fertiliser. Lorenz et al. (1994) also found a concomitant decrease in soil pH. Both the increase in soil solution Cd concentration and decrease in soil pH could be explained in part by desorption of ions from exchange sites on soil colloids. Mitchell et al. (2000) showed that increased ionic strength rather than pH was more important with regard to Cd phytoavailability. In a growth chamber experiment Mitchell et al. (2000) showed that the application of N fertiliser up to 800 µg

Table 4 Correlations (r) between grain cadmium (Cd) concentration and grain and total biological yield (TBY) for two durum wheat (*Triticum turgidum*) cultivars at three sites.

Site	Cultivar	Grain yield	TBY
Darfield	CRDW17	0.89 $P < 0.05$	0.97 $P < 0.01$
	Waitohi	0.85 $P < 0.05$	0.95 $P < 0.01$
Wakanui	CRDW17	–0.20	0.56
	Waitohi	0.70	0.93 $P < 0.01$
Lincoln	CRDW17	0.78	0.78
	Waitohi	0.64	0.68
All sites and cultivars		0.60 $P < 0.001$	0.76 $P < 0.001$

N g^{-1} as urea led to increases in ionic strength, Cd concentration in soil solution, and increased plant uptake of Cd, but little effect on soil solution pH.

Given that the form of N applied to the wheat crop in this investigation was CAN, which has a negligible equivalent acidity, it is unlikely that the soil pH would have decreased significantly from CAN application during the period of the trial. An alternative explanation for the increase in Cd in grain with increasing N application is through increased root interception or enhanced mass flow, resulting from the increase in TBY with N application. The TBY of a wheat crop can be related directly to the amount of water used. For durum wheat and bread wheat the water use efficiency is c. 550 g water g^{-1} DM produced (Hay & Walker 1989). Therefore crops that produce higher TBY also use a greater amount of water. Assuming a constant soil solution Cd concentration within each of our experiment sites (Table 2), the crops that produced the highest TBY could be expected to have greater uptake of Cd. There is some evidence to support this in the present study. Specifically where TBY was significantly increased by N fertiliser there was a corresponding increase in grain Cd concentration. For example at Darfield, N increased TBY in both cultivars and a significant correlation with grain Cd concentration was found (Table 4). Similarly, the correlation between grain Cd and TBY was the same or stronger than with grain yield for both cultivars at the Wakanui and Lincoln sites.

This stronger correlation with TBY than grain yield is consistent with the known mechanisms of Cd uptake and translocation through a plant. The translocation of Cd from root to shoot has been investigated for a number of plant species including durum wheat (e.g., Jalil et al. 1994). Hart et al. (1998) suggested movement of Cd from roots to shoots is likely to occur via the xylem and to be driven by transpiration through plant leaves. Support for this was shown in the study by Salt et al. (1995) who showed that Cd accumulation in shoots of Indian mustard was dramatically reduced when stomata were induced to close. It follows then that plants which have a higher TBY, and consequently greater transpiration and water flow, can be expected to have a greater uptake of Cd. Translocation of Cd from soil solution to grains may then be related to phloem-mediated Cd transport (Hart et al. 1998). Variation in HI (Table 3) would then be expected to cause a difference in the grain Cd concentration as a result of differences in the proportion of grain filling from current and stored assimilate. Understanding of the mechanisms of grain filling and Cd transport are necessary before prediction of grain Cd concentration and explanations of the conflicting results in the literature in the response of grain Cd concentrations to grain yield (e.g., Jones & Johnston 1989; Grant et al. 1996; Mench et al. 1997; Gray et al. 2001) can be made.

CONCLUSIONS

The application of increasing amounts of CAN fertiliser increased Cd concentrations in two durum wheat cultivars grown at three different sites. In most instances, both the highest yield and grain Cd concentrations were associated with the highest application of N fertiliser. It is suggested that the higher yielding wheat crops had higher water use requirements, and consequently that greater volumes of water and solutes including Cd were moved from the soil to the root zone and translocated to the grain of the plant. Also important may be changes in the ionic composition of soil solution with increasing N application. It may be that when ammonium and Ca ions are in excess at periods during crop growth, Cd is desorbed from soil colloids into soil solution and hence available for plant uptake. Further work is required to determine the processes that lead to increased Cd uptake through the addition of N and other fertilisers to soils.

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