THE EFFECTS OF FARM MANAGEMENT PRACTICES ON CADMIUM CONCENTRATION IN WHEAT GRAIN

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SUMMARY

Cadmium is a heavy metal that is not known to have any essential biological function in plants or animals. There has recently been concern that Cd concentrations in some Australian food products may exceed the maximum permissible concentration (MPC) set by the Australian National Health and Medical Research Council (NHMRC) for the general category of 'unspecified foods', namely 0.05 mg kg^{-1} fresh weight. Thus, in Australia the concern about Cd in wheat grain has arisen from a marketing and trade viewpoint.

In Australia, the main source of Cd in agricultural soils is phosphate fertilizers. Although the use of phosphatic fertilizers containing low Cd concentrations will minimise the input of Cd into the agricultural system it will not solve the problem of Cd uptake by grain because of the accumulation of residual Cd in the plough layer (0-10 cm). Many factors control the availability of Cd in soil for plant uptake. Among the most widely documented are soil pH, clay content and form, organic matter content and Zn concentrations in the soil. This study has focussed on the effect of the following factors on Cd concentrations in wheat grain: Zn nutrition, crop rotations, tillage practices, and soil pH in field and glasshouse experiments. The effectiveness of soil extraction procedures to correlate Cd concentration in grain and extractable soil Cd was also investigated.

Grain and soil samples were collected from thirteen field experiments located across South Australia and four field experiments located in southern Queensland. The Zn concentration in the soils at all sites was determined to be inadequate for crop growth prior to the establishment of the experiments. Zinc was applied as zinc sulphate at rates ranging from 0-20 kg Zn ha^{-1} The grain was analysed for Cd concentration and the soil samples (0-10 cm) were analysed for pH and EDTA-extractable Cd and Zn concentrations. Grain from the South Australian experiments was also analysed for

other metals using inductively-coupled plasma (ICP) analysis. This study showed that applications of Zn (approximately 2.5 - 5.0 kg ha^{-1}) to soils that had inadequate Zn concentrations for crop growth decreased the Cd concentrations in grain by up to half at some sites. The Cd concentrations in grain from the Queensland experiments were near the detection limit which made it difficult to assess any trends in Cd concentrations with increasing rates of Zn application.

Grain and soil samples were obtained for 2 years (1986 and 1987) from a rotation x stubble x nitrogen experiment at Tarlee, S.A. and for 2 years (1989 and 1990) from a rotation x tillage x fertilizer experiment at Kapunda, S.A. In the Tarlee experiment, wheat is grown every year in two phases in each of eight rotations with cereals, legumes or pasture. The three stubble treatments are stubble burning, incorporation and surface retention. Nitrogen is applied as ammonium nitrate at 0 and 40 kg ha^{-1}. In the Kapunda experiment, wheat is grown annually in a continuous wheat rotation and every second year a wheat/volunteer pasture and a wheat/lupins rotation. The three tillage systems are direct drill, reduced till and conventional cultivation. The two fertilizer treatments are low (15 kg P and 40 kg N ha^{-1}) and high (30 kg P and 80 kg N ha^{-1}).

Grain from both experiments was analysed for Cd concentrations and soil was analysed for pH and EDTA-extractable Cd. Soil from the Tarlee experiment was also analysed for $Ca(NO_3)_2$ - extractable Cd. In both years of the Tarlee experiment and the 1990 harvest of the Kapunda experiment the highest Cd concentrations in grain were found in wheat grown after lupins and generally the lowest were in wheat grown after cereal. No explanation can be offered for the observation of higher Cd concentrations in wheat grain grown after lupins.

The Cd concentrations in grain increased significantly with increasing rates of nitrogen in both the 1986 and 1987 harvests of the Tarlee experiment. However, stubble treatment had no significant effect on the Cd concentration in grain in either year. Fertilizer treatments had no significant effects on Cd concentrations in grain from the Kapunda experiment.

Raising soil pH is the most commonly recommended practice to minimize Cd uptake by plants. In this study, the interaction between soil type, applied Cd and soil pH was investigated in a glasshouse experiment. The pH of four soils, which varied in physical and chemical characteristics, was adjusted with sulphur or lime to give a range of 4 - 6 (1:5, 0.01M $CaCl_2$). Cadmium was applied in a sand matrix to give a range from 0 - 2.7 mg Cd kg^{-1} soil. Cd concentrations in grain and plant material were determined on all replicates and soil pH, EDTA-, $CaCl_2$- and $Ca(NO_3)_2$-extractable Cd concentrations were measured on one-half of the replicates.

Raising soil pH decreased Cd concentrations in grain grown on three of the four soils used in the glasshouse experiment (a Typic Rhodoxeralf, a Natric Palexeralf and a Mollic Palexeralf). However on a Sodic Haploxerert, the Cd concentration in grain did not change significantly with increasing soil pH. The Cd concentrations in grain were found to decrease following the addition of S at the highest Cd treatment (2.7 mg Cd kg^{-1}) on the Sodic Haploxerert.

The effects of soil pH on Cd concentration in grain were also assessed in the field. Grain and soil samples were obtained from eight experiments in the Wagga Wagga, NSW and Rutherglen, Vic. regions. Sulphur and lime had been added to adjust soil pH to give a range from 4 - 6. Cadmium concentrations in grain, soil pH and EDTA-extractable Cd were measured on one-half of the replicates.

The responses of Cd concentrations in grain to raising soil pH in field experiments was extremely variable between sites and between seasons at individual sites. Consequently, the common recommendation of raising soil pH to decrease Cd

concentrations in grain is not always valid and further studies are required to determine the factors that influence whether a decrease in Cd concentration of grain will occur following the application of lime under field conditions.

Three extraction procedures were studied to assess the correlation between Cd concentration in grain and extractable soil Cd with the view of using an extraction procedure for predicting Cd concentrations in wheat grain. The extractants were EDTA, $Ca(NO_3)_2$ and $CaCl_2$. These three extractants were chosen because they have been used by many other researchers but seldom compared, and they extract different fractions of soil Cd. Generally there was a poor relationship between EDTA-extractable Cd and Cd concentrations in grain. The only exception to this was the regression between Cd concentration in grain and EDTA-extractable Cd for each individual soil used in the glasshouse experiment when all Cd treatments were considered. The positive correlation was most likely due to the wide range of soil Cd concentrations covered.

There was a good relationship between $CaCl_2$- and $Ca(NO_3)_2$-extractable Cd and Cd concentrations in grain grown on two soils in the glasshouse experiment (Mollic Palexeralf and Natric Palexeralf). The correlation for both extractants was poor for plants grown on the Sodic Haploxerert, which was most likely due to the absence of any change in Cd concentration in grain with increasing pH of this soil. Of the three extractants studied, $CaCl_2$ and $Ca(NO_3)_2$ showed the best correlations with Cd concentrations in grain. However, more work is required with a wider range of soil types to ascertain the effectiveness of the extractants to predict Cd concentrations in grain.

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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CHAPTER 1

INTRODUCTION

Cadmium is an element that is not known to have any essential biological function. However the recently increased industrial use of Cd has been implicated as a factor contributing to some general human and animal health problems (Schroeder, 1965; Carroll, 1966). Cadmium accumulates mainly in the liver and kidney of animals and humans and the accumulation in the kidneys reaches its maximum when a person is around 50 years old and then declines with increasing age. Symptoms of Cd toxicity in humans are initially vomiting, diarrhoea and colitis, while continuous exposure causes hypertension, heart enlargement and death (Friberg *et al.*, 1974; Rudd, 1987).

Despite the potential toxicity of Cd to humans there have been very few reported cases and those that have been reported have been the result of industrial contamination. The most well known case was the development of Itai-itai disease in Toyama Prefecture, Japan. The average Cd concentration (wet weight) in 20 samples of rice (*Oryza sativa* L.) from the endemic region was 0.68 mg kg^{-1} while the average in about 200 samples from other areas in Japan was 0.066 mg kg^{-1} (Friberg *et al.*, 1974). There have been no reported cases of Cd toxicity in agricultural regions that have not been contaminated by industrial pollution.

Generally, the introduction of Cd into the food chain is the result of contamination of soil with Cd from extraneous sources. In agricultural systems, the main sources of Cd are atmospheric pollution from the mining and smelting of Cd and Zn, the application of sewage sludge to land or the application of phosphate fertilizers that contain Cd as an impurity derived from the raw materials (Tiller, 1989). Although some agricultural areas, such as Port Pirie, South Australia, are contaminated with Cd due to atmospheric pollution from mining activities (Tiller *et al.*, 1975), the main source of Cd in Australian soils is the application of phosphate fertilizers (Tiller, 1989).

The amount of Cd in phosphate fertilizers (Table 1.1) is determined by the amount of Cd in the primary phosphate rock (Williams and David, 1973). The transfer of Cd from the phosphate fertilizer to the food chain is dependent upon the nature and amount of fertilizer used, its Cd content, the properties of the soil and the ability of the crop to absorb Cd (Smilde and van Luit, 1983).

Table 1.1 Cd concentrations in some phosphate rocks (mg kg^{-1})

	Source of phosphate rock	**Cd (mg kg^{-1})**
Australasia		
	Nauru*	70,89
	Christmas Island*	42
	Ocean Island*	99
	Duchess mine, Queensland	0.5, 4, 7
	Lady Annie mine, Queensland 7	
North America		
	Florida	20, 4, 7, 14
	North Carolina	21, 36
	Tennessee	0.1

*guano-derived phosphate deposit

From Cook and Freney (1988).

Australian fertilizers generally contain between 25 and 30 mg kg^{-1} Cd (Williams and David, 1973). Australian phosphate fertilizers have previously been produced from Nauru phosphate rock, which contains high Cd concentrations (Table 1.1). All high analysis and some low analysis phosphate fertilizers in Australia are now manufactured mainly from rock phosphates that are low in Cd (M. J. McLaughlin, pers. comm.). The use of low Cd rock phosphates minimises further additions of Cd to the soil but does not address the problem of the accumulation of residual Cd in the soil from previous phosphate fertilizer applications.

The Food Standards Committee of the National Health and Medical Research Council (NHMRC) in Australia has established the maximum permissible Cd concentration (MPC) in "unspecified foods", which includes wheat and wheat products except bran, at 0.05 mg kg^{-1} (NHMRC, 1987). The NHMRC have acknowledged that Cd accumulates in the aleurone layer of the grain and have set the MPC for bran at 0.2 mg kg^{-1}.

The limits for the MPC were determined by considering the toxicity of the metal under consideration, the rates of accumulation in the body, especially in the key organs where the metal accumulates, and the range of likely intakes. In the case of Cd, the key organ is the kidney. The Cd concentrations in food analysed in Market Basket Surveys and other surveys of food consumed by the population and estimations of the quantities of certain food groups ingested over specified time periods were taken into consideration.

There have been suggestions that the MPC limit for Cd in food in Australia may be too low and unrealistic for several reasons such as the assumptions made about the eating habits of the population and the uncertainty about the assimilation of Cd in the body. For example some researchers have suggested that binding factors in bran, such as dietary fibre and phytic acid, may prevent or decrease the absorption of Cd by the gastrointestinal tract. However studies of the effects of these binding factors on Cd assimilation in the body are inconclusive (Morberg *et al.*, 1987).

A recent study by the FAO/WHO (1990) indicated that the Australian population has one of the lowest dietary intakes of Cd of those countries surveyed. Nevertheless, there has been increasing concern in Australia about the Cd concentrations in food.

The concern is due to the low MPC set in Australia compared with those in other countries, and the potential trade repercussions.

In some of Australia's wheat (*Triticum aestivum* L.) growing regions the Cd concentration in wheat grain has exceeded the MPC . Although it is possible in some circumstances to blend grain from different regions and thus dilute the overall Cd concentration in the bulk sample, this practice is limited when wheat is exported to speciality markets which require a particular wheat variety or specific wheat qualities. Under these circumstances it may be more appropriate to manipulate management practices to minimise Cd accumulation in wheat grain.

The proposed research reported in this thesis used glasshouse experiments, established field experiments and laboratory studies to assess the effectiveness of specific management practices that could be manipulated to minimise Cd uptake. In particular this study addressed the following objectives:

1. The effects of the amelioration of Zn deficiency on the Cd concentration in wheat grain were determined.
2. The effect of different crop rotations, stubble handling practices and application of nitrogen on Cd concentration in wheat grain was assessed.
3. The effectiveness of raising soil pH to minimise grain Cd concentration of wheat was investigated.
4. Several soil extraction techniques were compared as methods for predicting Cd concentration in wheat grain to provide a basis for minimizing Cd concentration in grain where necessary.

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

Cadmium was first discovered by the German chemist, F. Strohmeyer, in 1817 which is quite recent in comparison with the discovery of Cu, Pb, Zn and Fe (Page *et al.*, 1981). In the environment Cd is always associated with Zn and the only known Cd minerals are greenockite (sulfide) and octavite (carbonate), which are both found as minor constituents associated with sphalerite (ZnS) and smithsonite ($ZnCO_3$) (Considine, 1976).

Cadmium occurs in quite low concentrations in Australian soils. Williams *et al.* (1972) analysed 19 Australian soils and found that the Cd concentration ranged from 0.02 to 0.38 mg kg^{-1}. The concentration can be increased by the application of sewage sludge, atmospheric pollution from smelters or by the application of fertilizers that contain Cd as a contaminant. The latter is the most common source of Cd in Australian agricultural soils (Williams and David, 1973). The concentration of Cd in phosphatic fertilizers is dependent on the Cd concentration in phosphate rock (Williams and David. 1973).

Although Cd uptake by plants has been extensively studied, particularly in Europe and North America, there has been little research into Cd concentrations in cereals in Australia. Further, the overseas research is not always applicable to Australia because of differences in Cd sources, differences in soil Cd concentrations, differences in environmental conditions, and differences in farm management practices. To minimise Cd concentrations in wheat grain by farm management practices, it is necessary to understand the soil processes that control the adsorption of Cd to soil particles. Thus, this review will discuss the major processes that affect Cd adsorption, the supply of Cd for plant uptake and the impact of farm management practices on Cd availability. Finally the use of soil tests to predict Cd concentration in wheat grain will be reviewed.

2.2 FACTORS CONTROLLING Cd CONCENTRATION IN SOIL SOLUTION

The concentration of Cd in soil solution and hence availability of Cd for plant uptake is influenced by:

1. sorption reactions with soil surfaces,
2. occlusion into soil minerals,
3. precipitation with other compounds in soils, and
4. presence of ligands capable of complexing Cd.

These processes in turn are controlled by numerous factors such as soil pH, ionic strength and the presence of other ions. These processes will be reviewed in the following sections.

2.2.1 Effect of pH on Cd Concentration in Soil Solution

Cadmium ions are adsorbed onto numerous soil surfaces including organic matter and sesquioxides. Soil pH affects Cd adsorption by influencing Cd speciation and surface charge on the adsorbing surfaces. In soils, the hydrous oxides of Fe and Mn may adsorb significant proportions of some metal ions. The Mn oxides have a high selectivity for certain metals such as Pb^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+}. Although the pH-dependence of metal adsorption would suggest that metal ions are retained by direct coordination to surface oxygen ions, for some metals the process is more complex. It has been suggested that oxidation of Co^{2+} could promote the subsequent adsorption of Co because of the greater affinity of Co^{3+} for oxide surfaces without actual substitution into the structure (McKenzie, 1967). The Fe oxides are of similar particle size to Mn oxides with correspondingly high surface area, but they are more abundant than Mn oxides. Therefore it is likely that the Fe oxides adsorb greater proportions of some metal cations, but not Co^{2+} or Pb^{2+} (Jones and Jarvis, 1981).

There are four main mechanisms involved in the pH-dependence of metal adsorption:

1. metal precipitation,
2. metal hydrolysis followed by adsorption,
3. competition of metal cations with protons for exchange sites, and
4. metal adsorption as determined by surface charge.

Metal precipitation

Both metal precipitation and adsorption processes may involve bonding of metals to oxides and hydroxides and it is sometimes difficult to make a distinction between metal adsorption and metal precipitation.

Transition and heavy metal solubility in soils is generally not considered to be controlled by the solubility of a pure solid phase. At the low concentrations of most metals in soil solutions, adsorption processes are able to maintain solubility at a level too low for precipitation to occur (Brummer *et al.*, 1983; Herms and Brummer, 1984 cited in McBride, 1989). Usually it is only under situations of high concentrations of metals in soil with low sorption capacity and high soil pH that precipitation occurs. Although there is evidence that $CaCO_3$ may control the concentration of Cd in solution in calcareous soils, the concentration of Cd in acidic soils is far below that predicted from the solubility of $CdCO_3$ or $Cd(OH)_2$ (Cavallaro and McBride, 1978).

Brummer *et al.* (1983) showed that the concentration of metals in solution could not be explained from solubility diagrams. Similarly Basta and Tabatabai (1992c) found major differences in metal adsorption among soils under various cropping systems and concluded these differences were most likely due to adsorption and not precipitation.

Metal hydrolysis

Hydrolysis reactions have also been put forward to explain the pH dependence of metal adsorption (Hodgson *et al.*, 1964). In the natural environment Cd occurs in the divalent state. In the presence of water metal ions can undergo hydrolysis reactions (Hodgson *et al.*, 1964; Lindsay, 1972) as shown below:

$$M^{2+} + H_2O \quad \leftrightarrow \quad MOH^+ + H^+$$

$$MOH^+ + X^- \quad \leftrightarrow \quad XMOH$$

where M^{2+} is the divalent metal cation and X is the adsorbing surface.

These equations show that hydrolysis is favoured by increasing the pH and is characterised by a sigmoidal adsorption vs solution pH curve (Forbes *et al.*, 1976; Tiller *et al.*, 1984). The hydrolysis products are more strongly adsorbed than the divalent free metal cations. This is thought to be due to the formation of hydroxy-complexes that are thermodynamically more stable and easier to adsorb than the free metal cation (James and Healey, 1972). The pH at which metals hydrolyse is dependent on their respective pK values and on the pH range of soils. The pK value for Cd is 10.1 (Baes and Mesmer, 1976). Transition and heavy metals possess a much greater tendency than alkaline earth metals to hydrolyse because of their ionic structure. Consequently, the formation of hydrolysis products has been suggested as an explanation for the greater specificity in adsorption of layer silicate clays for transition and heavy metals at higher pH values (McBride, 1989).

Competition between ions

The third mechanism to explain the pH dependence of metal adsorption is competition between protons and metal ions for cation exchange sites, which can be described as:

$$M^{2+} + HX \quad \leftrightarrow \quad M^+X + H^+$$

Other cations such as Ca^{2+}, Cu^{2+}, Zn^{2+} may compete with Cd for adsorption sites on soil surfaces. The hydrated ions of Cd^{2+} can react with negatively charged clay colloids as shown below

$$Cd^{2+} + CaX \quad \leftrightarrow \quad Ca^{2+} + CdX$$

Since the concentrations of Ca^{2+} ions in many soil solutions are relatively high (Loneragan, 1975) the left hand side of the equation would be favoured. This however is dependent on soil conditions such as pH and equilibrium constants. It has been suggested that the effect of liming on Cd adsorption by soils is in part due to competition between Ca^{2+} and Cd^{2+} ions. Tyler and McBride (1982b) found significant decreases in the Cd concentration of snap bean (*Phaseolus vulgaris* L.) foliage when grown in solution culture containing a higher level of Ca (0.005M $CaSO_4$ compared to 0.001M $CaSO_4$) for every pH treatment. Cavallaro and McBride (1978) found much decreased adsorption of Cd onto an acidic silt loam and a neutral silt loam in the presence of 0.01M $CaCl_2$ compared with Cd sorption when no $CaCl_2$ was added. Competing ions such as Ca^{2+} were found to shift adsorption equilibria for Cd^{2+} greatly, with less effect on Cu^{2+}. This implied that ion exchange was responsible for Cd^{2+} adsorption but Cu^{2+} may be bound more strongly to adsorption sites. However, the addition of Ca in the chloride form in this study may have affected the results since the chloride ion is able to form stable complexes with Cd (Tyler and McBride, 1982b).

Since Cd and Zn are both group IIB elements and Cd is strongly associated with Zn in its geochemistry, Zn ions have also been suggested as competitors with Cd for adsorption to soil surfaces. However Zn and Cd have different ionic radii (Zn^{2+} 0.074 nm and Cd^{2+} 0.097 nm). Cadmium also has a stronger affinity than Zn for S and is more mobile than Zn in acidic environments (Kabata-Pendias and Pendias, 1984).

Tiller *et al.* (1979) studied specific and non-specific sorption of Cd by various soil clays. Four clays were shaken for four days in a Ca nitrate suspension at pH 6.0 containing 10^{-5}M or 5.0 x 10^{-5}M Zn. They found addition of Zn decreased the specific adsorption of Cd in three of the four clays. However, for one clay the addition of Zn had no effect on the specific adsorption of Cd. This clay had the highest amount of adsorbed Cu and Zn, particularly when expressed on the basis of surface area. It was argued that the high affinity sites on the surfaces of this clay were already occupied by Cu and Zn so that the added Zn in the Ca nitrate suspension was ineffective in desorbing Cd.

An increase in the specific sorption of Zn by all four clays did not result in an equivalent decrease in Cd sorption. Tiller *et al.* (1979) suggested that either each metal ion has a very high preference for particular sites which are quite specific for that metal or both metals react with similar sites but by reactions of a variable but limited reversibility (Tiller *et al.*, 1979).

Christensen (1987a) similarly studied Cd adsorption on a range of soils in the presence and absence of a mixture of Ni, Co and Zn at two pH values. For all soils studied, the addition of a mixture of Ni, Co and Zn decreased the distribution coefficient, which is the slope of the equilibrium isotherm, although the slope remained linear. Thus, in the presence of the other metals there was less Cd adsorbed onto the soils than at the same concentration of Cd alone. The initial solute concentrations of Ni, Co and Zn were 300 $\mu g\ L^{-1}$, 120 $\mu g\ L^{-1}$, and 1500 $\mu g\ L^{-1}$ respectively, which were representative of a polluted soil. Zinc was found to account for most of the competition with Cd, since the isotherm for Cd and Zn corresponded to the isotherm for Cd and the mixture of Ni, Co and Zn.

The concentration of the competing metals was also found to be important because as the concentration of the mixture of Ni, Co and Zn decreased the amount of Cd adsorbed at each single concentration increased (Christensen, 1987a).

2.2.2 Adsorption Models

Several models, including the Langmuir and Freundlich equations, have been used to describe Cd adsorption reactions. An outline of these adsorption equations is given below.

Langmuir Equation

The Langmuir equation was originally developed to explain gaseous adsorption on a planar surface (Langmuir 1918) and is often adapted to describe solid-solution reactions in soils. The equation for adsorption of Cd from solutions to soil surfaces is:

$$q = (q_m k_c C) / (1 + k_c), \text{ or in the linear form } C/q = (1/kq_m) + (C/q_m)$$

where q is the amount of Cd adsorbed per unit of soil, q_m is the maximum amount of Cd that can be adsorbed onto a monolayer, C is the equilibrium Cd concentration in solution, and k_c is a constant related to bonding energy.

The main postulates of the Langmuir equation are (Adamson 1967; Bohn *et al.*, 1985):

1. the energy of adsorption is constant (which implies uniform adsorption sites and no interaction between adsorbate molecules);

2. the adsorption is on localised sites i.e. no translational motion of adsorbed molecules in the plane of the surface;

3. maximum adsorption possible corresponds to a complete monomolecular layer.

It has been shown however (Adamson 1967; Moore 1972) that many of these assumptions are not valid. For example, more than one type of adsorption site exists; the surface can be covered by multilayers; adsorbed molecules can move laterally on the surface; there is interaction between adsorbate molecules; and adsorption energy as well as probability of adsorption or desorption is dependent upon proportion of bonding sites occupied.

The limitations of the application of the Langmuir equation to soil-solution systems has been acknowledged by many others (e.g. Griffin and Au, 1977; Harter and Baker, 1977; Veith and Sposito, 1977). Despite these limitations the Langmuir equation has been used by several researchers to describe Cd adsorption to soil surfaces (John, 1972; Riffaldi and Levi-Minzi, 1975; Cavallaro and McBride, 1978; Navrot *et al.*, 1978; Christensen, 1987a and 1987b).

For example, Christensen (1987b) used a Langmuir approach to explain the competitive effects of Zn on Cd distribution assuming both metals adsorb onto the same sites through surface complexation. In an earlier study, Christensen (1987a) had shown that in the absence of other metals the Cd adsorption isotherms approached linearity.

He stated that Zn competition with Cd for adsorption sites depends on the shape of the Zn adsorption isotherm but not the shape of the Cd sorption isotherm. Hence, in this model the ability of a metal to compete for adsorption sites depends on both the soil adsorption stability constant and the solute concentration of the competing metal.

It would appear that the Langmuir equation has been adopted because it happens to be a good empirical equation and not because the specified assumptions have been obeyed. Harter and Smith (1981) suggested that the Langmuir equation may be useful to characterise the initial instantaneous reaction that occurs too rapidly to follow by kinetic studies. However they argued that the Langmuir equation is inadequate for the entire reaction because adsorption reactions at the soil-solution interface are too complex and many other reactions account for ion retention by soils.

Quirk and Posner (1974) modelled the adsorption of specifically adsorbing ions by placing them in another layer some distance from the potential determining ions, which were regarded as being in the Stern layer of the surface. The equation they used took into account all specifically adsorbing species present in solution including hydrolysed cations but excluding potential determining ions. They found their model gave a better fit to experimental data than a simple Langmuir plot. Their model also allowed for a change in binding energy as the charge on the surface varied, either through a change in pH or adsorption, which is not allowed for in the simple Langmuir model.

Adsorption isotherms

The plot of q, the amount of solute or metal adsorbed per unit of soil, as a function of C, the Cd concentration in solution, may often be sufficient to determine an adsorption maximum provided the amount of Cd adsorbed reaches a plateau. This approach however has been criticised because the solution concentrations necessary to complete such an isotherm are frequently considered unrealistic compared to those found in nature (Harter and Smith, 1981).

Freundlich equation

The Freundlich equation may be written

$$X = kC^b$$

where k and b are 'linear' and 'exponential' coefficients, respectively. Adamson (1967) showed the Freundlich equation corresponds to a model of adsorption in which the affinity term decreases exponentially as the amount of adsorption increases. Garcia-Miragaya and Page (1976) found adsorption of low concentrations (15 to 120 $\mu g\ L^{-1}$) of Cd to montmorillonite followed the Freundlich sorption isotherm.

Arrhenius equation

The Arrhenius equation (Harter and Smith, 1981) is

$$(d \ln (k_r))/ dT = Ea/RT^2$$

where k_r is the reaction rate constant, T is the absolute temperature, R is the gas constant and Ea is the Arrhenius activation energy. This equation can be integrated to the form

$$k_r = A \exp(-Ea / RT)$$

where A is a constant known as the frequency factor. The plot of $\ln k_r$ as a function of 1/T with the intercept (1/T = 0) will give the constant A and the slope will be equal to -Ea/R. Determining Ea is important since it indicates the size of the energy barrier that must be overcome for the reaction to proceed and indicates the reversibility of the reaction. However, in the normal time frames (several weeks to years) that most researchers study chemical reactions between soil and trace elements it is often difficult to measure the reversibility of these reactions.

2.2.3 Metal Adsorption by Organic Matter

Metal bonding to organic matter can be viewed as an ion exchange process between H^+ and metal ions on acidic functional groups. However, the high degree of selectivity of organic matter for certain metals strongly suggests that some metals form inner-sphere complexes with the functional groups. In general, the more electronegative metal ions bond most strongly to organic matter. The 'driving force' for metal adsorption can be the higher covalency of the metal-organic bond relative to the metal-water bond. There is also the opportunity for multidentate coordination of the metal (e.g. chelation) which provides additional stability for metal-organic complexes (Jones and Jarvis, 1981; McBride, 1989). In general, the stability of the complexes increases with increasing soil pH due to increased ionisation of functional groups (Jones and Jarvis, 1981).

2.2.4 Metal Occlusion into Minerals

Sorption of heavy metals onto Fe- and Al-oxides is an inner sphere complexation that does not obey the reversible mass action relationships predicted for simple cation exchange. This implies that metals chemisorbed by inner-sphere complexation are unlikely to be very exchangeable by cations that have no specific affinity for the oxide. Gerth and Brummer (1981) used this to explain the decreased ability of strong acids to reverse adsorption of Ni^{2+}, Zn^{2+} and Cd^{2+} onto oxides, such as goethite, with time. McBride (1989) stated that true solid diffusion (i.e. diffusion within the crystal compared with diffusion between crystals) is unlikely to occur since the structure would be destabilised by the penetration of divalent metals below the surface layer of the oxide and a more likely process is very slow diffusion of metals into extremely small pores of particle aggregates.

Whilst the mineralogical composition of the soil, the presence of soluble organic ligands, and pH largely control the solubility of heavy metal ions other factors may sometimes be important such as soil temperature, the stability of the Cd complexes (Page *et al.*, 1981; Alloway, 1990), microbiological activity and redox potential (Jones and Jarvis, 1981).

2.3 EFFECT OF SOIL pH ON Cd CONCENTRATION IN PLANTS.

Increasing the soil pH by liming has been recommended by some researchers as an effective method of decreasing the mobility of Cd and decreasing the uptake of Cd by plants (Page *et al.*, 1981; Alloway, 1990). To minimise Cd uptake from soils ameliorated with sewage sludge the United States Environmental Protection Agency (USEPA) used to specify that the pH_W of the soil should be maintained at or greater than 6.5 (USEPA, 1978). However more recent legislation has removed this requirement (USEPA, 1993).

Although there has been much research into the effects of soil pH on Cd uptake by plants, most of the research has been with soils which had high metal concentrations following the application of sewage sludge and the results have been contradictory. While Cd concentrations in plants have been found to decrease with increasing pH in some studies (Andersson and Nilsson, 1974; Street *et al.*, 1978; Bingham *et al.*, 1979; Allinson and Dzialo, 1981) other studies have found no change or an increase in Cd concentration with increasing pH (Maclean, 1976; Pepper *et al.*, 1983; Eriksson, 1989).

Numerous researchers have investigated the effect of soil pH on Cd uptake in glasshouse experiments. Street *et al.* (1978) found increasing the pH_w of a sand from 5.7 to 7.8 decreased the Cd concentration in maize (*Zea mays* L.) seedlings by 70% when Cd was added as $CdSO_4$ to give 3.0 and 6.0 mg Cd kg^{-1} of soil. Similarly, raising the soil pH_w from 4.5 to 6.4 significantly decreased the concentration of Cd, Pb and Ni in ryegrass (*Lolium hybridum* L.) when grown in a glasshouse experiment in succession with oats (*Avena sativa* L.) (Allinson and Dzialo, 1981). Andersson and Nilsson (1974) found at any application rate of sewage sludge to a loamy sand the Cd concentration in fodder rape (*Brassica napus* L.) decreased with increasing pH. Further, liming decreased the concentration of Cd in wheat grain by 50% when grown in sewage sludge spiked with metal sulphates (Bingham *et al.*, 1979).

In some studies however, the effect of soil pH on Cd uptake has been variable. Maclean(1976) found additions of lime to acidic sandy soils in a pot experiment decreased the Cd concentration in lettuce (*Lactuca sativa* L.), although the results were inconsistent. Pepper *et al.* (1983) limed sludge-amended field plots to raise the soil pH_{Ca} from 4.6 to 6.3 at two sites. They found no significant decrease in Cd concentration in maize leaves following liming. At sludge loadings greater than 22.4 t ha^{-1} the leaf Cd concentration was greater on limed plots than on unlimed plots on a fine sandy loam. In that study the sludge was probably controlling Cd availability and soil processes will only control Cd availability in a few years following the last sludge application. In soils of low reactivity the Ca^{2+} from sludge may compete with Cd^{2+} for adsorption sites. Consequently it is difficult to draw comparisons between experiments involving sewage sludge applications and those involving the application of the metal as a salt.

Eriksson (1989) similarly found the Cd concentration of ryegrass plants grown in a clay soil varied between pH treatments in a complicated manner. Raising the pH_w of the clay soil from 5 to 6 by adding CaO increased the Cd concentration in ryegrass while further additions of CaO to increase the pH_w to 7 decreased the Cd concentration. For all Cd treatments (0, 1.0 and 5.0 mg Cd kg^{-1}) in the clay soil decreasing the soil pH_w from 5 to 4 by the addition of sulphur resulted in either no change or an unexpected decrease in the Cd content of ryegrass. In contrast, there was a consistent decrease in Cd concentration in ryegrass with increasing pH_w of a sandy soil.

It has been suggested that the occasional increase in Cd concentration in plants observed after small amounts of CaO had been added to soil was caused by the added Ca removing exchangeable Cd from the soil colloids and thereby rendering it temporarily more available to plants (Eriksson, 1989). As the soil pH increased, adsorption of Cd would be partly irreversible and Ca^{2+} would be unable to compete as strongly for adsorption to inorganic colloids (Jones and Jarvis, 1981).

In a study with tobacco (*Nicotiana tabacum* L.), King (1988) found additions of lime to nine different soils resulted in significant decreases in the Cd concentrations of the lower leaves for only three of the soils. However, in this glasshouse experiment three of the soils (two Histosols and an Enceptisol) were still acidic (pH_w 4.9 to 5.5) despite liming, which may explain the absence of a decrease in Cd concentration with increasing rates of lime. However no significant decrease in Cd concentration were found on two other soils when the pH_w was increased from 5.6 to 6.4 and 6.2 to 7.0, respectively. The author suggested that the high organic matter content of the non-responsive soils may explain the result. However, a high organic matter content would be expected to adsorb Cd and plants grown in these soils would be expected to take up less Cd than those grown in soils with a lower organic matter content.

Eriksson (1990) conducted a comprehensive survey of Cd concentrations in oat and wheat grain collected across Sweden. He does not record whether any of the sites had received sludge. However the total soil Cd concentration ranged from 0.071 to 0.62 mg kg^{-1} which would suggest that the sites had not received sludge. Although he found a strong negative relationship between Cd concentration in oat grain and soil pH, only 24% of the variation was accounted for by soil pH. He also found a negative correlation between Cd concentration and soil pH for wheat grain but there is no information about the amount of variation accounted for by soil pH.

Hydrogen ions have also been claimed to depress Cd uptake by plants by competing with Cd ions. Hatch *et al.* (1988) studied Cd uptake by perennial ryegrass (*Lolium perenne* L.), cocksfoot (*Dactylis glomerata* L.), lettuce and watercress (*Rorippa nasturstium-aquaticum* L.) in flowing solution culture experiments (pH 5-7) containing Cd at the concentration of 0.018 mM m^{-3}. They found the total uptake of Cd was lowest from nutrient solutions at pH 5.0 and increased by factors of four in cocksfoot, eight in perennial ryegrass and ten in lettuce and watercress as the pH of the nutrient solution increased to 7.0. In contrast, Tyler and McBride (1982b) found in similar solution culture experiments (pH 4-7) with seedlings of snap beans and maize to which Cd was added as $Cd(NO_3)_2.4H_2O$, that pH had only a small effect on the Cd uptake by maize. There was some evidence of inhibition of absorption and translocation at low pH. From this study they stated the hydrogen ion did not compete effectively with the Cd ion for uptake and the pH of the solution surrounding the roots had no direct effect on uptake of Cd. Hatch *et al.*(1988) claimed that the discrepancy between their results and those of Tyler and McBride (1982b) was due to the latter using high concentrations of Cd in solution that may have obscured the competitive effect of hydrogen ions.

There is only limited information in the literature about the effects of raising soil pH on Cd concentrations in plants grown in the field in soils with low Cd concentrations. The use of sewage sludge and/or elevated soil Cd concentrations makes it difficult to transpose results from overseas studies to Australian soils. In this study more information will be obtained about the effects of pH on Cd concentrations in wheat grain grown in the field in soils that have not received any sludge and have comparatively low Cd concentrations.

2.4 EFFECT OF Zn ON Cd CONCENTRATION IN PLANTS

The similarity in the chemical behaviour of Cd and Zn in soils has been noted by many researchers, and consequently there have been many studies on the effects of Cd on Zn concentrations in plants and vice versa. However the majority of researchers (Haghiri, 1974; Maclean, 1976; Bingham *et al.*, 1979; White and Chaney, 1980) have investigated soils with high Cd and/or Zn concentrations in the soil. Further the results have been contradictory. While some researchers (Lagerwerff and Biersdorf, 1972; Haghiri, 1974) have found a synergistic effect between the two metals on plant uptake, others have shown inhibitory effects or no effects at all (Maclean, 1976; Singh and Steinnes, 1976; White and Chaney, 1980). The variability in results may be due to the particular plant species grown or plant fraction analysed, the reactions occurring between the two elements in the substrate or the relative concentrations of Zn and Cd used.

For example, Maclean (1976) used five physically and chemically different soils in a pot experiment to study the interaction between Cd and Zn. Both metals were added to the soils as chloride salts at a concentration of 5 mg kg^{-1}. In nearly all cases the addition of Cd to the soil increased the concentration of Zn in lettuce plants. However, added Zn did not affect the Cd uptake. Haghiri (1974) conducted a similar experiment with soybeans (*Glycine max* L.). Cadmium in the form of $CdCl_2$ was incorporated into a silty clay loam soil at a concentration of 10 mg kg^{-1} (soil basis). Zinc was then incorporated into the soil as $Zn(NO_3)_2$ solution at concentrations of 0-400 mg kg^{-1} (soil basis). The addition of Zn from 5-50 mg kg^{-1} significantly increased the Cd concentration in soybean shoots as compared with the control, but at 100 mg Zn kg^{-1} a decrease in Cd concentration relative to the control began to occur. He suggested that the increase in Cd concentration in plant tissue by the addition of 5-50 mg Zn kg^{-1} was due to decreased plant growth and possibly increased displacement of Cd into the soil solution from the soil exchange complex. Further, he explained the depression in Cd concentration in soybean shoots in treatments 100-400 mg Zn kg^{-1} to be the result of higher concentrations of Cd than Zn in the soil solution due to excess amounts of Zn being present. It seems unlikely that the higher concentrations of Zn in soil solution would increase adsorption of Cd onto soil fractions, but rather that the higher Zn concentration in the soil solution would enable Zn to compete more effectively for uptake by the plant.

While some studies have shown that Zn and Cd interact synergistically in relation to plant uptake, others have found no relationship at all. White and Chaney (1980) found the Cd concentration of soybean leaves remained unchanged irrespective of the Zn treatments when grown on a fine loamy, mixed thermic soil containing a high organic matter and low Fe- and Mn-oxide content. Although a similar result was observed when soybeans were grown in a fine loamy, mixed mesic soil the foliar Cd concentrations increased at the higher Zn additions (> 393 mg kg^{-1}). This is in direct contrast to Haghiri's (1974) work with soybeans where the Cd concentration in the shoots decreased at the higher Zn concentrations (100-400 mg Zn kg^{-1}). Similarly, the concentration and uptake of Cd by barley (*Hordeum vulgare* L.) plants grown in Zn-polluted soils in a greenhouse was independent of Zn treatments added to the soil as $ZnSO_4.7H_2O$ (0-600 mg kg^{-1}) (Singh and Steinnes, 1976).

In comparison to these experimental observations a number of other researchers (Hawf and Schmid, 1967; Bingham *et al.*, 1979) have observed competitive effects between Cd and Zn in relation to plant uptake. For example, Bingham *et al.* (1979) found applications of Zn (25, 100 and 200 mg kg^{-1}) to both an unlimed and limed fine sandy loam resulted in consistent decreases in the Cd content of grain.

However, only a few studies have been conducted to investigate the effects of amelioration of Zn deficiency on Cd concentration in plants (Honma and Hirata, 1978; Abdel-Sabour *et al.*, 1988; Gupta and Potalia, 1990). Abdel-Sabour *et al.* (1988) found Zn applications (0-50 mg kg^{-1}) generally decreased Cd concentrations of tops of maize and Swiss chard (*Beta vulgaris* L.). Gupta and Potalia (1990) similarly found Cd concentrations in wheat grain decreased with increasing Zn applications (0 - 20 mg Zn kg^{-1}) to a sandy soil (pH 8.5) in a glasshouse experiment.

In summary, while some workers have found that Zn and Cd interact competitively in plant uptake others have found either no interaction or a synergistic interaction between the two metals. Some workers have also noted competition between Zn and Cd for adsorption sites while others have found no interaction. The majority of investigations have used soils with relatively high concentrations of either one or both metals. The present study focuses specifically on the effect on Cd concentration in wheat grain of Zn applications to Zn-deficient soils.

2.5 EFFECT OF OTHER IONS ON Cd CONCENTRATION IN PLANTS

Other inorganic ions that have been shown to affect Cd availability to plants include chloride, carbonate and sulphate ions. Cadmium complexes readily with chloride and in saline soils a significant proportion of the Cd in the soil solution could be expected to be complexed by chloride ligands (Hahne and Kroontje, 1973; Bingham *et al.*, 1984). For example, Bingham *et al.* (1984) found salinization of a fine sandy loam to bring chloride concentrations in the saturation extract to 5, 15, 30, 45 or 60 mol m^{-3} increased Cd concentrations in the soil solution. Cadmium concentrations in the leaves of Swiss chard increased under the combined high Cd and high $CaCl_2$ treatment.

2.6 THE EFFECT OF CROP ROTATIONS, TILLAGE PRACTICES AND STUBBLE HANDLING METHODS ON Cd CONCENTRATION IN WHEAT GRAIN

Crop rotations, tillage practices and stubble handling methods tend to be specific to countries and even to regions within countries. These farm management practices can have significant effects on nutrient uptake by crops due to their effects on the physical characteristics of the soil and hence the soil micro-environment, which may affect root growth. They can also influence the distribution of nutrients within the soil profile and the chemical form of nutrients and hence their availability for plant uptake. The effects of crop rotations, tillage practices and stubble handling methods on Cd uptake are discussed separately in the following sections.

2.6.1 Crop Rotations

It was not until early this century that serious experimentation with crop rotations began. Rotations based on pasture-wheat or pasture-fallow-wheat were practised in Australia until the 1960s (Pratley and Rowell, 1987). The decline in productivity of Australian soils, particularly in marginal areas, mainly through repeated cropping and lack of fertilizer, led to experimentation with different crop rotations, which led to the inclusion of peas, rapeseed and lupins in rotations (Poole, 1987).

Crop rotations can affect the Cd taken up by the following crop in two main ways. The previous crop(s) can affect soil chemical and physical properties. These can in turn influence the availability of Cd for plant uptake and can affect the concentration of Cd in the root zone through inherent differences in the concentrations of Cd accumulated in different plant shoots and roots.

Legume-based pastures generally give improvements in soil structure and nitrogen concentrations (Greenland, 1971) and cereals grown after grain legumes have shown increased yields which may be due in part to enhanced nitrogen supply.

The increased content of soil nitrogen due to legumes in crop rotations also affects soil pH. Plants take up nitrogen as an anion (nitrate, NO_3^-), as a cation (ammonium, NH_4^+) or as a neutral nitrogen molecule in nitrogen fixation. To maintain charge balance during the uptake process, H^+ or OH^- ions must pass out of the root into the surrounding soil. While the uptake of NH_4^+ results in a net release of H^+ ions, uptake of NO_3^- can result in a net release of OH^- ions. In the case of nitrogen fixation the neutral nitrogen can be assimilated into protein and no charge imbalance is generated across the soil/root boundary (Helyar, 1976; Bolan *et al.*, 1991). However, many legumes commonly export H^+ ions into the rhizosphere when actively fixing nitrogen (Nyatsanga and Pierre, 1973). Although no ionic species of nitrogen are taken up by the plant during nitrogen fixation, basic cations, such as Ca^{2+} or K^+, are imported into the legume in exchange for H^+ ions generated during carbon assimilation. To maintain a pH balance these H^+ ions are exported from the roots. The amount of H^+ ions released during nitrogen fixation depends mainly on the form and amount of amino acids and organic acids synthesised in the plant (Helyar, 1976; Bolan *et al.*, 1991).

Decreasing the soil pH generally increases the availability of Cd for plant uptake as discussed in Section 2.2. The effect of growing a legume crop in a single season on soil pH would most likely be negligible, since the protons in the rhizosphere would diffuse in the soil.

A legume-based pasture grown for several seasons may be expected to have a greater acidifying effect on the soil pH than a legume crop grown for one year. Williams (1980) found the pH of a soil under permanent pasture had decreased one unit in 30 years. If the pH at the establishment of the pasture was around pH_{Ca} 5.5-6.0 or lower, then a decrease in soil pH would have significant effects on the availability of Cd for plant uptake.

In addition to crops affecting rhizosphere pH and thus Cd availability for the following crop, species and cultivars within species will differ in their ability to accumulate Cd which may also affect Cd availability for the following crop. Lettuce and certain other leafy vegetables have been shown to concentrate Cd (Haghiri, 1973; Purves and MacKenzie, 1973; Giordano *et al.*, 1979). John (1973) found large differences in Cd concentration in plants grown on a silt loam to which 40 mg Cd kg^{-1} and 200 mg Cd kg^{-1} had been added. The highest Cd concentrations in leaves were found in lettuce and radish (*Raphanus sativus* L.) at both Cd treatments. Maclean (1976) similarly grew a range of plants in a loam soil to which Cd as $CdCl_2$ (0, 2.5 and 5.0 mg Cd kg^{-1}) was added. He found the addition of Cd increased the Cd concentration in all plant parts analysed. In both studies (John, 1973; Maclean, 1976) there were also large differences in Cd concentrations between species grown in control plots.

Merry and Tiller (1991) found a five-fold difference in Cd concentration between subterranean clover (*Trifolium subterraneum* L.) and capeweed (*Arctotheca calendula* (L.) Levyns), a common annual weed, in pastures near Adelaide, South Australia.

Although many of the species which are grown in rotation with wheat in Australia were not analysed in the studies by John (1973) and Maclean (1976) it is clear that some plants accumulate more Cd than other plants. The use of "accumulator" plants to remediate polluted soils has been suggested by other researchers (Chaney, 1983; Baker *et al.*, 1986; Schlegel *et al.*, 1991). For example, Baker *et al.* (1986) found that the grass, *Holcus lanatus*, was able to grow in soils containing high Cd concentrations. Their results suggested that Cd tolerance is inducible and can be lost by transplanting to uncontaminated soils. Chaney (1983) suggested some plants, such as *Thlaspi* spp., are capable of accumulating Zn and Cd and could be used for biological remediation of soils contaminated with high concentrations of metals. However, this is not a practical option for broad scale agriculture and has only been considered for highly contaminated sites.

Although a pasture phase is included in some areas in the rotation sequence, the use of capeweed as an "accumulator" plant is not encouraged. Where the capeweed component of a pasture is not controlled capeweed can provide a significant Cd intake for grazing animals, particularly sheep (Merry and Tiller, 1991). The subsequent increase in the concentration of Cd in sheep offal has had serious trade implications.

Other than adsorption studies with soils from different long rotation experiments (Basta and Tabatai, 1992a, b and c) there is very little information about the effects of different cropping sequences on Cd concentration in wheat grain or indeed in any other plants. More information is required about Cd accumulation in different plants commonly grown in rotations and the effects of these crops on the Cd concentration in plants grown in subsequent years. This is particularly important in countries like Australia, which have low MPC for Cd in food products, since the sequence of crops can easily be manipulated by farmers. However the factors such as disease control, weed control and economics must also be taken into account. More information is also needed about varietal differences in Cd accumulation so that varieties which accumulate low concentrations of Cd can be grown in regions with high soil Cd concentrations.

2.6.2 Tillage Practices

Development of conservation-farming techniques acknowledges that frequent cultivation leads to a decline in soil organic matter, loss of soil aggregation, higher soil strengths, compacted plough layers and smaller soil faunal populations (Poole, 1987). Although the benefits of minimum tillage where cultivation has been almost removed from the crop production system is well recognized, more than 90% of soils sown to cereals in southern Australia still receive some cultivation prior to crop establishment (Pratley and Rowell, 1987).

Tillage can affect the availability of Cd to plants by affecting i) the amount and distribution of roots within the soil; ii) the amount and distribution of water and nutrients in soil and iii) the abundance and activity of soil fauna.

Tillage can increase, at least temporarily, surface storage of water by roughening the surface. The effect of tillage on runoff, relative to an untilled surface, is to increase potential rates of infiltration through the creation of macropores, which in turn increases the effective surface area for infiltration. The breaking up of surface crusts may also be important. These effects are short-term since slumping and crusting often develop with further rain. However, deep tillage can provide long-term positive effects by breaking up discrete natural or tillage-derived sub-surface barriers. This has led to deeper rooting and greater water uptake from the deeper layers of soil (Taylor, 1974).

Barriers to root movement through the soil profile, such as impermeable hard pans, may confine roots to the upper layers of soil. Since phosphatic fertilizers tend to be cultivated through the surface layers ($\cong$ 10 - 15 cm depth for cereals) in Australia the restriction of root growth to the upper soil horizons, and hence the zone of highest Cd concentration is likely to increase Cd uptake by plants. In direct drilled situations the rate of root extension and shoot growth have been found to decrease when soil strengths in the surface soil have increased (Poole, 1987). Thus, after direct-drilling, the Cd uptake by wheat may be expected to be more than by wheat grown in a conventional cultivation system due to the restriction of root exploration to the surface layers.

There is little information in the literature about the effects of tillage on Cd concentrations in any plant. However, by comparing the effects of tillage practices on the concentrations of other metals in plants it is possible to predict how these practices may affect the Cd concentration in wheat grain.

Tillage practices will also affect nutrient distribution within the soil profile. Mixing of surface-applied nutrients may increase the extent of reactions between applied nutrients and inorganic constituents of soil, thus decreasing the availability of nutrients to plants. Alternatively, mixing may also increase the amount of contact between roots and applied nutrients thereby increasing the availability of nutrients to plants. The relative magnitude of these two opposing effects determines the effect of tillage on nutrient uptake by plants.

Williams (1977) found significantly higher Cd concentrations in subterranean clover grown in pots when the Cd was mixed through the soil compared with Cd applied only to the surface. Similarly, Gartrell (1981) found cultivating soil to which Cu had been added greatly increased the Cu concentration in barley leaves. Cultivation of the soil increased the contact between the roots and Cu resulting in increased concentrations. Cadmium from phosphatic fertilizers is immobile in soils and tends not to be leached out of the "plough" layer (≅ 10-15 cm for cereals). Cultivation may also greatly increase Cd uptake by plants as a result of the increased distribution of Cd through the soil and thus increased contact between roots and Cd.

Tillage methods may also influence soil pH and therefore Cd uptake as detailed in earlier sections. Tillage methods, crop rotations and stubble handling practices affect soil pH by influencing:

1. the amount and distribution of organic matter,
2. the transformation of nitrogen in soil,
3. the uptake of cations relative to anions by plants, and
4. the return of cations relative to anions in plant residues.

Tillage practices can affect the variation in soil pH, distribution of organic matter and extractable nutrients with depth. The absence of mixing by cultivation in untilled soils may lead to acidification of one soil layer and/or the alkalization of another (Robson and Taylor, 1987). Frequently, non-tilled soils are more acidic in the surface 7.5 cm and less acidic in deeper layers than ploughed soils (Shear and Moschler, 1969). However, nitrification, whereby NH_4^+ is oxidised to yield NO_3^- and protons are produced, is often slower in the surface of non-tilled soils (Robson and Taylor, 1987).

As discussed in Section 2.2, decreasing the soil pH generally increases the availability of Cd for plant uptake so any tillage practices that acidify the soil would be expected to increase Cd uptake. Under a direct-drill system Cd uptake could possibly be increased due to increased acidity in the surface layer which have a higher Cd concentration than deeper in the soil profile, and greater root exploration in the surface layers.

Studies are required to assess long term effects of tillage practices on nutrient availability as well as the effects on Cd concentration in wheat grain.

2.6.3 Stubble Handling Practices

Exposure of bare soil surface to rain and traffic causes slaking and dispersion of a much larger proportion of the surface than occurs under continuous plant cover

(Hamblin, 1987). Thus, the aim of stubble retention techniques is to retain stubble on the soil surface for as long as possible to protect the surface from wind and water erosion. However, stubble burning or removal has continued because of problems with crop establishment and nutrition, poor stubble-handling capacity of the combine drill and problems related to management of disease, weeds and insects (Poole, 1987).

Residue mulching has been shown to decrease soil movement and runoff substantially compared with residue incorporation or residue burning (Freebairn and Wockner, 1982, cited in Holland *et al.*, 1987). A major practical benefit of retaining residues is increased soil water content near the soil surface due to increased infiltration, and decreased evaporation in situations where rainfall occurs regularly (Felton *et al.*, 1987). Increased soil water content near the soil surface increases germination rate, seedling survival and restricts root exploration, at least during the early stages of growth, to the surface (Hamblin, 1987). Consequently, Cd uptake should increase during early growth stages because the root system would be extending only in the surface horizons which is the region of highest Cd concentration in the soil profile.

The presence of plant residues on the soil surface can also significantly influence soil temperature. Surface mulches reduce the net radiation input to the soil surface and have an insulating effect resulting in smaller diurnal soil temperature variation. Johnson (1990) found that Cd adsorption onto goethite in solution increased with temperature. This effect was found at different pH values over the range 6-9, for an initial Cd concentrations of 10^{-4}M. Thus mulching may decrease Cd adsorption to soil fractions as compared to bare soil, by lowering the soil temperature.

At higher soil temperatures plant metabolic activity increases, movement of ions in the soil are increased (Nielsen, 1974) and the reaction rates of metals with inorganic ligands is increased (Lindsay, 1972). Giordano *et al.*(1979) heated soil in the field using electric resistance cables to maintain temperature differentials of about 6, 8 and

11°C above ambient temperature at 7.5, 15 and 30 cm depths in the early spring. Sewage sludge was applied to soil one year prior to sowing various vegetable crops. They found heating the soil doubled the concentration of Cd in the edible parts of broccoli (*Brassica oleracea* L.). No significant increases in metal concentrations were seen in either the edible parts or foliage of lettuce, eggplant (*Solanum melongena* L.), tomato (*Lycopersicon esculentum* Mill.) or potato (*Solanum tuberosum* L.). Sheaffer *et al.* (1979) similarly grew maize in the field on a sandy loam to which sludge was applied. Four temperature regimes (16, ambient ($\cong$ 22°C), 27 and 35°C) were maintained from sowing through to harvest. Significantly ($P<0.05$) lower concentrations of Zn in maize grain and Cu in maize ear leaves and stover were observed at 16°C than at the higher temperatures. The effects of temperature on Cu, Cd, Ni and Pb concentration were less pronounced than for Zn and more variable. Possibly lowering soil temperature by maintaining stubble on the soil surface may decrease the Cd concentration in wheat plants.

Stubble left on the surface of soils is likely to decompose more slowly than that incorporated into soil mainly because of lower water content in stubble on the surface (Robson and Taylor, 1987). Decreased decomposition will lead to less organic material in the surface layers and potentially fewer adsorption sites for Cd. Conversely, increased decomposition of stubble would increase soil organic matter resulting in potentially more binding sites for Cd. However, the adsorption of Cd onto organic material will also be affected by tillage practices and the activity of micro-organisms involved in the degradation of organic material. Stubble may also contain Cd that was accumulated during the previous growing season which may become available for plant uptake when the organic material containing the Cd has decomposed. The increase in metal availability due to decomposition of organic matter has been observed by others. For example, Giordano *et al.* (1979) observed increased DTPA - extractable Cd, Zn, Cu, Pb and Ni from 1975 to 1977 from soil that had received sewage sludge in 1975. They stated the increased metal availability was due to the progressive decomposition of the organic matrix.

In summary, crop rotations, tillage practices and stubble handling methods will interact to affect Cd concentration in plants through their effects on physical and chemical soil factors. Crop rotations, tillage practices and stubble handling methods may influence root extension and exploration of the soil profile and the form of Cd in the soil and hence its availability for plant uptake. In this review the conclusions about the effects of crop rotations, tillage practices and stubble handling methods on Cd concentration in wheat grain have been speculative due to the limited amount of information available about the effects of these farm management practices on the availability of Cd and other elements for plant uptake. This indicates the need for further research in this area to understand how changes to farm systems affect Cd concentration in grain.

2.7 DETERMINATION OF PLANT - AVAILABLE Cd

Many series of sequential extractants have been used to estimate the percentage of the metal(s) in soil associated with designated soil fractions e.g. "easily-exchangeable", "inorganically bound" and "organically bound". Sequential extraction procedures have recently been reviewed by Beckett (1989) but this review will be restricted only to extractants that aim to estimate concentrations of metals, particularly Cd, available for plant uptake.

For such soil tests to be effective the techniques must be simple and rapid, and provide a good correlation with concentrations of the metals in plants across a range of soil types and metal concentrations in the soil. Frequently soil tests for predicting Cd concentration in plants have only been tested across a narrow range of soils. This is most likely due to cost and time involved in such a survey.

Ammonium Acetate

Some researchers (Symeonides and McRae, 1977; Kiekens *et al.*, 1984; Eriksson, 1989) have used ammonium acetate buffered at a set pH to determine plant available fractions of metals. For example Eriksson (1989) found in a glasshouse study high correlations between the Cd concentration in ryegrass and rapeseed grown in a range of Cd (0-5 mg kg^{-1}) and pH (4-7) treatments on two soils and the soil Cd extracted with 1M ammonium acetate (pH 4.8) and that extracted with 1M ammonium acetate (pH 7.0). He stated the high correlation mainly reflected that both the Cd concentration of plants and Cd in soil extracts increased in direct proportion to the amount of Cd added to the soil. Further, variations in Cd concentration of plants caused by differences in soil pH were not easily predicted by measuring extractable Cd since Cd in the ryegrass and rapeseed were not influenced in the same way by pH changes as was Cd in soil extracts. Kiekens *et al.* (1984) also studied Cd uptake by ryegrass grown on only two soils that had been amended with four rates of sewage sludge. They found that the Zn, Cu, Cd and Ni concentrations extracted by five different extractants gave significant correlations with the metal concentration in ryegrass. On a sandy soil the concentration of Cd extracted with both 1M ammonium acetate (pH 7.0) and water accounted for 99.1% and 99.5%, respectively, of the variation in plant Cd concentration. On a heavy clay soil, the Cd concentration extracted by both 1M ammonium acetate (pH 7.0) and ammonium acetate and EDTA (pH 4.65) accounted for 96% of the variation in ryegrass Cd concentration. Again the high correlation was most likely due to the Cd concentration in ryegrass and in soil extracts increasing in direct proportion to the increase in soil Cd following additions of sewage sludge.

Neutral Salts

Others have not favoured ammonium salts because ammonium and acetate ions may have specific complexing effects. For example, ammonium acetate was found to mobilise more organic matter than did 0.05M $CaCl_2$ (McLaren and Crawford, 1973). Although the extraction of metals under standard conditions (including pH) may be an advantage for some workers, others have found buffered reagents affect the pH at the exchange sites which will affect the amount of Cd extracted. Sauerbeck and Styperek (1985) advocated the use of unbuffered neutral salts to minimize change in H^+ ion concentration of soils. This is important since uptake of Cd by plants depends on its concentration in the soil solution. Consequently neutral salts such as chlorides or nitrates of K, Mg or Ca have been used to estimate "available" ions (Tiller *et al.*, 1972; Bjerre and Schierup, 1985; Sauerbeck and Styperek, 1985; Eriksson, 1990; Whitten and Ritchie, 1991; Smilde *et al.*, 1992) of trace metals from soils and sludge.

For example, Smilde *et al.* (1992) found a positive linear relationship for Cd and Zn concentrations in lettuce, spinach (*Spinacia oleracea* L.), spring wheat and maize grown on a sandy soil (pH_{KCl} 4.2) and a loam (pH_{KCl} 7.2) and 0.01M $CaCl_2$-extractable Cd and Zn. However, in their study only two soils with three Cd treatments (0-4.0 mg kg^{-1}) and five Zn treatments (0-80 mg kg^{-1}) were used and the relationship between plant Cd concentration and $CaCl_2$-extractable Cd was not well established for the low soil Cd concentrations (< 0.01 mg kg^{-1}). Whitten and Ritchie (1991) similarly found strong correlations between Cd concentration in the whole tops of subterranean clover and $CaCl_2$-extractable Cd concentrations ($r^2 = 0.91$). However only three soils were used in this study (pH_{Ca} 4-7) and all three soils were sandy with the sand content ranging from 80 - 90%.

One of the most extensive surveys conducted was by Eriksson (1990) who collected 204 oat grain and soil (0-20 cm) samples and 125 wheat grain and soil (0-20 cm) samples across Sweden. As recommended by Sauerbeck and Styperek (1985) he used 0.05M $CaCl_2$ (1:10) to extract Cd from the soils. He stated that $CaCl_2$-extractable Cd accounted for most of the variation in Cd availability. However his data did not indicate this. The inclusion of $CaCl_2$-extractable Cd in the regression model accounted for 42% of the variation in Cd concentration in oats but only 4% of the variation in Cd concentration in wheat. He claimed that the lack of correlation between $CaCl_2$-extractable Cd and Cd concentration in wheat was most likely due to the Cd concentration in wheat grain not being as strongly correlated with pH as that of oats. This suggests that $CaCl_2$ is not a good soil test for predicting grain Cd concentration.

A consequence of using chloride salts as extractants to estimate soil Cd available for plant uptake is the formation of stable complexes. The equilibrium constant for $Cd^{2+} + Cl^- \rightarrow CdCl^+$ is 10^3. Thus chloride salts would be expected to extract higher concentrations of Cd than nitrate salts of the same concentration. Cadmium extracted by $Ca(NO_3)_2$ may better approximate that in soil solution and that weakly bound to soil surfaces while the Cd extracted by $CaCl_2$ would shift the equilibrium from solid to soil solution because of the formation of complexes with chloride ions. Specifically adsorbed Cd would not be displaced by Ca ions of either extractant except from acidic soils. However, provided the Cd concentration in the extraction correlated well with the grain Cd concentration then either extractant should be a good soil test for predicting Cd concentrations in grain. In this study, the effectiveness of both $Ca(NO_3)_2$ and $CaCl_2$ extracts to predict Cd concentration in grain grown on soil containing low concentrations of Cd will be assessed.

EDTA and DTPA

Other researchers (Maclean, 1976; Symeonides and McRae, 1977; Latterell *et al.*, 1978; Merry and Tiller, 1978; Street *et al.*, 1978) have investigated stronger complexing agents such as ethylene diamine tetra-acetic acid (EDTA) or diethylene triamine penta-acetic acid (DTPA) as extractants. Beckett's (1989) review states the three main roles of EDTA are:

1. to dissolve carbonates,
2. to extract trace metals from the oxides or hydroxides of Fe, and
3. to release organically bound trace metals.

Hornburg and Brummer (1986) state that the EDTA- and DTPA-extractable Cd fractions characterise the total quantity of potentially available Cd in soils. Both EDTA and DTPA are much "stronger" extractants than the neutral salts and thus may not be considered very suitable extractants to predict metal availability for plant uptake.

However Merry and Tiller (1978) found EDTA (0.1M, pH 6.0)-extractable Cd accounted for 74%, 82% and 90% of the variation in Cd concentration in *Maireana* spp., Marrubium and *Medicago* spp., respectively. These plants were collected during a survey of a region in close proximity to a Pb-Zn smelter complex at Port Pirie, South Australia, on highly calcareous soils. The good correlation found by Merry and Tiller (1978) would be partly due to dust particles adhering to the surface of the foliar material collected since they made no attempt to remove adhering dust from leaf or stem surfaces. This was done in order to retain the material in a state similar to that ingested by grazing animals.

Street *et al.* (1978) grew maize in four soils which had either been amended with sludge or $CdSO_4$ and found that DTPA-extractable Cd accounted for 96% of the variation in the Cd concentration in maize tops. As with many other studies though the value of the work is limited by only four soils being used. In addition apart from one soil which had a pH_w of 5.71, the soils were mainly alkaline (pH_w 7.43 to 8.25). Latterell *et al.* (1978) found DTPA-extractable soil Zn and Cd accounted for 90% and 72% of the variation in the concentrations of Zn and Cd in edible tissue in snap bean leaves. Their experiment was conducted on only one soil, which had elevated Zn and Cd concentrations due to annual applications of sewage sludge.

By contrast Symeonides and McRae (1977) found 0.05N EDTA-extractable Cd accounted for only 29% of the Cd concentration in radish tops grown on 25 (0-25 cm) soils to which Cd had been added. Hornburg and Brummer (1986) similarly found no significant relationship between EDTA- and DTPA-extractable Cd in soils and Cd in wheat grain. They found other soil factors such as pH, EDTA- extractable Cu and Zn had to be taken into account to explain the variability of Cd concentration in wheat grain.

Thus many types of soil extractants have been used to examine the relationship between soil and plant Cd concentrations with varying degrees of success. Ideally a large number (>50) of soils should be used in such studies but this is often cost-prohibitive. The effectiveness of extractants to correlate the relationship between Cd concentration in soil and in wheat grain will be tested in this study. Soil tests could provide a method for screening sites prior to sowing and would allow farmers in areas with "high" soil Cd concentrations to implement management strategies to minimise Cd concentrations in wheat grain prior to sowing if necessary. This would ensure that their grain met the MPC for Cd concentration of 0.05 mg kg^{-1} set by the NHMRC (Australia). However for a soil test to be universally applicable it must meet the criteria stated earlier.

2.8 CONCLUSIONS

In conclusion, there are many varied and complex factors that affect Cd availability to plants. The different soil types, high soil Cd concentrations, usually arising from the application from sewage sludge, and different species of plants in the experimental work reported make it difficult to transfer overseas findings directly to Australian conditions. Furthermore, there appear to be discrepancies in the literature about Zn - Cd interactions and the effects of pH on Cd accumulation in plants. In this thesis, studies are conducted to assess the effectiveness of selected management practices available to farmers to minimise Cd accumulation in plants and to attempt to provide some recommendations about available strategies.

CHAPTER 3

THE EFFECTS OF ZINC FERTILIZATION ON CADMIUM CONCENTRATION IN WHEAT GRAIN

3.1 INTRODUCTION

The effects of Zn on Cd uptake by plants has been extensively studied in glasshouse and field experiments. However, results from these studies are conflicting. For example, the Cd content of lettuce (Maclean, 1976); barley (Singh and Steinnes, 1976) and soybeans (White and Chaney, 1980) has been found to be independent of Zn treatments. However, Haghiri (1974) found additions of Zn from 5 to 50 mg kg^{-1} to soil significantly increased the Cd concentrations of soybean shoots but at Zn applications >50 mg kg^{-1}, the Cd concentration in soybean shoots was decreased compared with the control. In contrast, Zn applications (0-50 mg kg^{-1}) to a Zn-deficient silt loam were found to decrease Cd concentration in Swiss chard and maize (Abdel-Sabour *et al.*, 1988). This result occurred with the application of both Zn sulphate and Zn acetate. Similarly Honma and Hirata (1978) found Zn applications (0.05 mg kg^{-1}) decreased the Cd concentration in rice roots and shoots when grown in solution culture compared with rice grown under Zn-deficient conditions. Hirth (1978) also found additions of $ZnSO_4.7H_2O$ to a self-mulching clay decreased Cd uptake by wheat tops. The largest decrease occurred with Zn applications of 1 - 2 kg Zn ha^{-1}.

This variability in results may be due to differences in the plant part or the plant species analysed. Furthermore, most studies on Cd-Zn interactions have focussed on soils containing elevated levels of Zn and/or Cd as a result of soil pollution or the application of sewage sludge to the soil.

Many soils in Australia have low concentrations of Zn, resulting in extensive areas where wheat plants are deficient or nearly deficient in Zn (Reuter *et al.*, 1989). For many decades single superphosphate contained Zn impurities which provided some of the Zn requirements of plants. More recently high-analysis fertilizers, containing negligible amounts of trace elements as impurities, have been used more extensively.

The decrease in the amount of Zn added as impurities in phosphatic fertilizers and changes in wheat crop varieties grown has led to an increased incidence of Zn deficiency and therefore an increased use of Zn supplemented fertilizers to ameliorate the deficiencies. In the following investigation the interaction between Cd and Zn under conditions of low soil Cd and Zn concentrations was examined with the aim of determining whether application of Zn is an effective management practice for minimising Cd concentrations in wheat grain.

3.2 MATERIALS AND METHODS

3.2.1 Field Experiments

South Australia

Grain and soil samples were collected from 13 field experiments established by the South Australian Department of Agriculture. The experiments were established across South Australia on soils with low Zn concentrations to investigate the rates of Zn required in different climatic regions and on different soil types to ameliorate Zn deficiency in wheat. All sites were marginally Zn deficient as evidenced by yield responses following applications of Zn at each site measured prior to the commencement of the experiment. Further, concentrations of DTPA-extractable Zn had indicated that the Zn concentration at the majority of sites was below the recommended critical value for adequate plant growth (Lindsay and Norvell, 1978). Five experiments were on Eyre Peninsula and four were in the Murray Mallee region (Fig. 3.1a). At each site the plot sizes were 1.8 m wide by 20 m long. Details of the experiments and descriptions of the soil at each site are given in Tables 3.1, 3.2a and 3.2b.

In all experiments the Zn treatments were completely randomised within four or six replicate blocks. Zinc, applied as Zn sulphate (0-20 kg Zn ha^{-1}), was sprayed onto the soil surface and mixed through the top 10 cm by rotary-hoeing. Results of the effects of Zn applications on crop yield in these experiments will be presented elsewhere (R. Hannam and N. Wilhelm, unpublished).

For all experiments, except Experiments 3.7, 3.8, 3.9 and 3.12, wheat grain analysed for Cd was harvested in the same year as the Zn was applied. The Zn treatments added in 1989 to Experiments 3.10 and 3.13 were applied to plots that did not previously receive Zn. These treatments are called "fresh" Zn applications. The treatments in Experiments 3.9 and 3.12 that were applied three and two years respectively, before the wheat harvest, are called "residual" Zn applications (Table 3.1).

Queensland

Grain of wheat cv. Hartog was also obtained from the 1989 harvest of experiments in southern Queensland. These experiments were established by the Queensland Department of Primary Industry (Toowoomba). Grain was provided from four sites near Toowoomba : Kupunn, Miles, Pilton and Kommamurra (Fig. 3.1b). Some characteristics of the soils at these sites are given in Table 3.3.

The plot sizes in these experiments were 28 m x 4.5 m for Experiments 3.14 and 3.15; 23 m x 5.6 m for Experiment 3.16 and 16 m x 6.8 m for Experiment 3.17. Zinc, applied as Zn sulphate (0 - 15 kg Zn ha^{-1}), was sprayed onto the soil surface and incorporated into the surface layers ($\cong$0-10 cm). The Zn treatments were completely randomised in four replicate blocks.

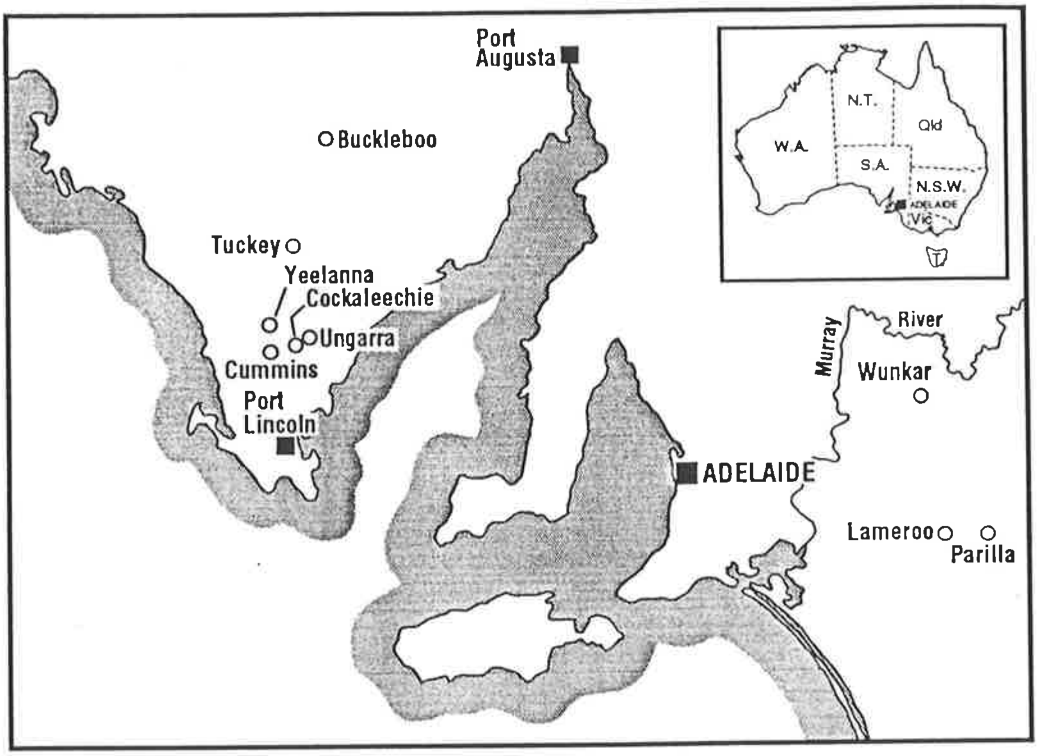

Figure 3.1a. Location of Zn fertilisation experiments in South Australia.

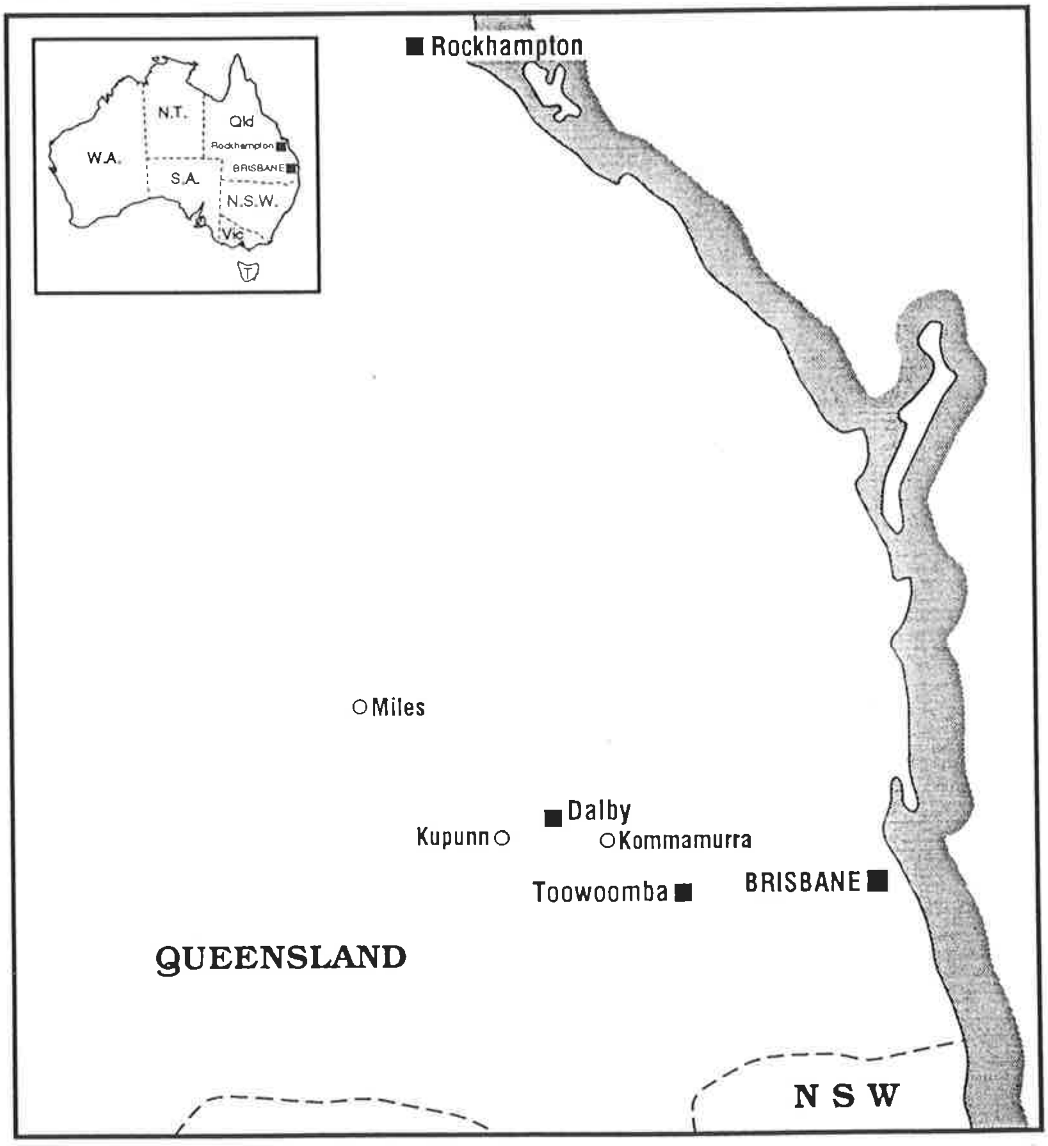

Figure 3.1b. Location of Zn fertilisation experiments in southern Queensland.

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TABLE 3.1 Details of South Australian experiments

Experiment	Location & Region	Reps	Year Of Zn Application	Wheat Variety	Harvest Analysed
i "Fresh" Zn					
3.1	Tuckey, EP[1] 136° 05' W, 33° 39' S	4	1988	Spear	1988
3.2	Buckleboo, EP 136° 04' W, 32° 48' S	4	1988	Spear	1988
3.3	Cummins, EP 135° 44' W, 34° 16' S	6	1988	Spear	1988
3.4	Wunkar, MM[2] 140° 18' W, 34° 29' S	4	1988	Spear	1988
3.5	Parilla, MM 140° 40' W, 35° 18' S	4	1986	Aroona	1986
3.6	Lameroo, MM 140° 31' W, 35° 20' S	4	1987	Aroona	1987
3.10	Ungarra, EP	4	1989[3]	Machete	1989
3.11	Yeelanna, EP 135° 44' W, 34° 09' S	6	1987	Spear	1987
3.13	Yeelanna, EP	6	1989[3]	Machete	1989
ii "Residual" Zn					
3.7	Lameroo, MM	4	1985	Aroona	1989
3.8	Ungarra, EP 136° 03' W, 34° 11' S	4	1986	Spear	1987
3.9	Ungarra, EP	4	1986	Machete	1989
3.12	Yeelanna, EP	6	1987	Machete	1989

[1] EP Eyre Peninsula

[2] MM Murray Mallee

[3] The Zn treatments ranged from 0-20.0 kg Zn ha^{-1} for all experiments except experiment 3.5 where the highest Zn rate was 25 kg Zn ha^{-1}.

Zn treatments added in 1989 to experiments 3.10 and 3.13 were applied to plots that did not previously receive Zn. These treatments are called "fresh" Zn applications.

TABLE 3.2a Classification and texture of soils from the South Australian experiments

Experiment	Soil Classification[1]	Soil Texture	Particle Size Clay %	Silt %	Sand %
i *"Fresh" Zn*					
3.1	Calcixerollic xerochrept	Sandy loam	12	4	81
3.3	Calcic palexeralf	Sandy clay	38	8	48
3.4	-	Sandy loam	12	5	78
3.5	Typic natrixeralf	Sand	2	1	94
3.6	Calcic palexeralf	Sand	5	1	91
3.10	Calcic natrixeralf	Sand	1	1	96
3.11	Calcic rhodoxeralf	Sandy clay loam	29	12	51
3.13	Calcic rhodoxeralf	Sandy clay loam	30	12	53
ii *"Residual" Zn*					
3.7	Calcic palexeralf	Sandy loam	13	2	81
3.8	Calcic natrixeralf	Sand	1	2	95
3.9	Calcic natrixeralf	Sand	0	1	96
3.12	Calcic rhodoxeralf	Sandy clay loam	32	13	52

[1] Soil Survey Staff, 1990

TABLE 3.2b Selected chemical characteristics of soils from the South Australian experiments

Experiment	pH[1] (1:5, 0.01M $CaCl_2$)	EDTA Zn (mean ± sd) (O Zn added)	EDTA Cd (mean ± sd) (O Zn added)
		mg kg^{-1}	
i *"Fresh" Zn*			
3.1	6.93 - 7.77	0.580 ±0.014	0.063 ±0
3.3	7.73 - 7.78	0.662 ±0.045	0.050 ±0
3.4	7.93 - 8.06	0.635 ±0.120	0.011 ±0.006
3.5	5.97 - 6.24	0.370 ±0.085	0.011 ±0.016
3.6	5.71 - 6.74	0.480 ±0.127	0.066 ±0.016
3.10	4.96 - 5.02	0.700 ±0	0.058 ±0.007
3.11	7.49 - 7.77	0.730 ±0.077	0.078 ±0.009
3.13	7.56 - 7.77	0.919 ±0.153	0.083 ±0.004
ii *"Residual" Zn*			
3.7	7.65 - 7.92	0.455 ±0.064	0.044 ±0.016
3.8	5.39 - 5.57	0.715 ±0.021	0.069 ±0.023
3.9	4.91 - 5.06	0.700 ±0	0.058 ±0.007
3.12	7.43 - 7.80	0.919 ±0.153	0.083 ±0.004

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[1] Range of mean values across treatments

TABLE 3.3 Selected texture and chemical characteristics of the soils from the Southern Queensland experiments

Experiment	Location	Soil Texture	% Clay	% Silt	% Sand
3.14	Kupunn	heavy clay	67	16	33
3.15	Miles	loam	39	12	49
3.16	Pilton	heavy clay	67	21	11
3.17	Kommamurra	heavy clay	65	17	18

Experiment	% Organic Carbon	pH (1:2, 0.01M $CaCl_2$)	Range of EDTA Cd (mg kg^{-1})
3.14	0.67	7.65	< 0.010 - 0.025
3.15	0.78	7.65	0.020 - 0.058
3.16	1.27	6.54	-
3.17	0.92	6.71	-

All soils were Vertisols (Soil Survey Staff, 1990).

3.2.2 Chemical Analyses of Grain

Cd

The digestion procedure was adapted from a method developed by the State Chemistry Laboratory in Victoria (Shelley, 1990). One gram of whole grain was digested in 8 cm^3 concentrated nitric acid and 1.5 cm^3 perchloric acid (70%). The digestion temperature was gradually increased up to 210°C and the grain was left to digest until the volume was reduced to 1 cm^3. Deionised water (18 cm^3) was added and the pH was adjusted to between 3-4 with ammonium acetate. The Cd was complexed with 0.5% ammonium pyrrolidine dithiocarbamate (APDC) and then extracted into 10 cm^3 of di-isobutyl ketone (DIBK). The solution was mixed thoroughly and allowed to separate for approximately 15 minutes. The aqueous phase was removed and the Cd was back-extracted into 10 cm^3 of 3% nitric acid. All samples were digested at least twice in separate batches.

The digested samples were allowed to stand overnight and, on the following day, aliquots of the 3% nitric acid extract were analysed by a graphite furnace atomic absorption spectrophotometer (AAS) (Varian SpectrAA-400) using a Varian hollow cathode Cd lamp. Measurements were made at wavelength 228.8 nm and background absorption was corrected with a hydrogen lamp. The detection limit for Cd was 0.002 mg kg^{-1} grain (Rothery, 1985).

All glassware was acid washed, rinsed in deionised water and oven-dried before use. Any Cd contaminants in the ammonium acetate were removed by cleaning with APDC and DIBK.

The reproducibility of the analytical procedure was checked by including our own standard grain samples in every batch of eighty samples. Two samples of wheat flour (National Bureau of Standards No. 1567a) were also included in every third batch.

The expected Cd concentration for this standard sample was 0.026 ± 0.002 mg kg^{-1} and the mean value obtained was 0.023 ± 0.003 mg kg^{-1}.

Analysis of grain for elements other than Cd

One gram of grain was digested in 10 cm^3 concentrated nitric acid at 200°C until the volume was decreased to 1 cm^3 (Zarcinas *et al.*, 1987). The digest was then made up to 20 cm^3 with deionised water and samples were analysed for Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn on the Labtest inductively coupled plasma spectrophotometer (ICP) (Zarcinas and Cartwright, 1983). ICP analyses were done on grain samples only from the South Australian experiments.

3.2.3 Soil Sampling and Analyses

Five 0-10 cm cores were collected each year prior to sowing the crop from plots in all of the South Australian experiments. The samples were bulked, sieved to pass a 2 mm mesh screen and mixed well before being sub-sampled for analysis. The soil from Experiment 3.2 was not studied because the experiment had been affected by drought and no significant effects of Zn treatments on the Cd concentration in grain were found. Soil samples were available from only two Queensland experiments (Experiments 3.14 and 3.15). Three 0-10 cm cores were collected in the year following harvest (i.e. 1990), bulked, oven-dried at 30°C and ground in a stainless steel mill. Sub-samples were taken for analysis.

Soil pH was measured following shaking soil samples (5g) with 25 cm^3 of 0.01M $CaCl_2$ for one hour. The pH of the supernatant was measured within an hour using a Metrohm 632 pH meter on all replicates from each experiment, except Experiment 3.2, and is termed pH_{Ca} in the text. pH measurements made in 1:5 soil:water extractions are termed pH_w.

Cadmium and Zn were determined in extracts after shaking 10g soil samples with 25 cm^3 of 0.05 M ethylene diamine tetra-acetic acid (EDTA) at pH 6 for 7 days at 20°C (Clayton and Tiller, 1979). Before shaking the samples for 7 days carbon dioxide was allowed to evolve from calcareous soils by shaking the sample, releasing the stopper and allowing the sample to stand for 2 hours. The solutions were analysed on a flame AAS for Cd and Zn. Half of the replicates of each experiment were extracted with EDTA.

3.2.4 Statistical Analyses

The effects of Zn treatments on grain Cd concentrations were determined using analyses of variance. Cadmium concentrations were fitted to an exponential model (Payne *et al.*, 1989). Statistical differences between the curves were determined by comparing the estimates and parameters summed across the two curves under consideration with the pooled estimates and parameters for the two curves (Ratkowsky, 1983). An F-value was determined from the ratio of the variance of the difference with the variance of the sum, which were calculated as follows:

$$\text{variance of sum } (MS_{sum}) = (\Sigma_{1,2}\text{Residual SS})/(\Sigma_{1,2}\text{Residual df})$$

and

$$\frac{\text{variance of difference}}{(MS_{difference})} = \frac{(\text{pooled Residual SS}) - (\Sigma_{1,2}\text{ Residual SS})}{(\text{pooled df}) - (\Sigma_{1,2}\text{ Residual df})}$$

where subscripts 1,2 refer to the two curves being compared, MS = mean square, SS = sum of squares and df = degrees of freedom.

Details of the exponential equations for the relationship between grain Cd concentration and Zn application and the adjusted R^2 values for each experiment are given in Table 3.4. The Zn application at which half the observed decrease in Cd concentration ($ODCd_{50}$) was obtained, was calculated by (log 2/ k) (Table 3.4).

3.3 RESULTS

3.3.1 Effect of Zn Fertilization on Cd Concentration in Wheat Grain

Applications of Zn to the soil significantly decreased the Cd concentration in the wheat grain at most sites (Figs. 3.2a - 3.2d). Exponential models have been fitted to all the data for each experiment and only the mean concentration for each rate of Zn application are shown. Since generally the grain Cd concentrations in all experiments except Experiments 3.8 and 3.11 showed no further decreases with Zn applications greater than 10 kg Zn ha^{-1} no data for higher Zn treatments are presented. The exponential curves for Experiments 3.8 and 3.11 continued to decrease with increasing Zn rates and therefore data for Zn rates above 10 kg Zn ha^{-1} are presented.

The grain Cd concentrations in Experiments 3.1, 3.6 and 3.7 decreased sharply with increasing Zn applications up to 2.5 kg Zn ha^{-1}. At 2.5 kg Zn ha^{-1} the concentrations were almost halved, decreasing from approximately 0.040 to 0.020, 0.045 to 0.023 and 0.025 to 0.012 mg kg^{-1} for Experiments 3.1, 3.6 and 3.7 respectively (Fig. 3.2a and 3.2c). There was no statistical difference between the two exponential curves for Experiments 3.1 and 3.6 but the curves for Experiments 3.5 and 3.3 were statistically different. The interpretation of the curves for Experiments 3.1 and 3.6 and Experiments 3.3 and 3.5 is difficult due to variation between experiments in the harvest analysed and the variety grown (Table 3.1). However, the $ODCd_{50}$ was obtained at approximately the same Zn rates for Experiments 3.5, 3.6 and 3.7 (Table 3.4). The Cd concentrations in Experiment 3.5 were low (<0.02 mg kg^{-1}) in grain from the control treatment and were not affected significantly by the additions of Zn fertilizer (Fig. 3.2a).

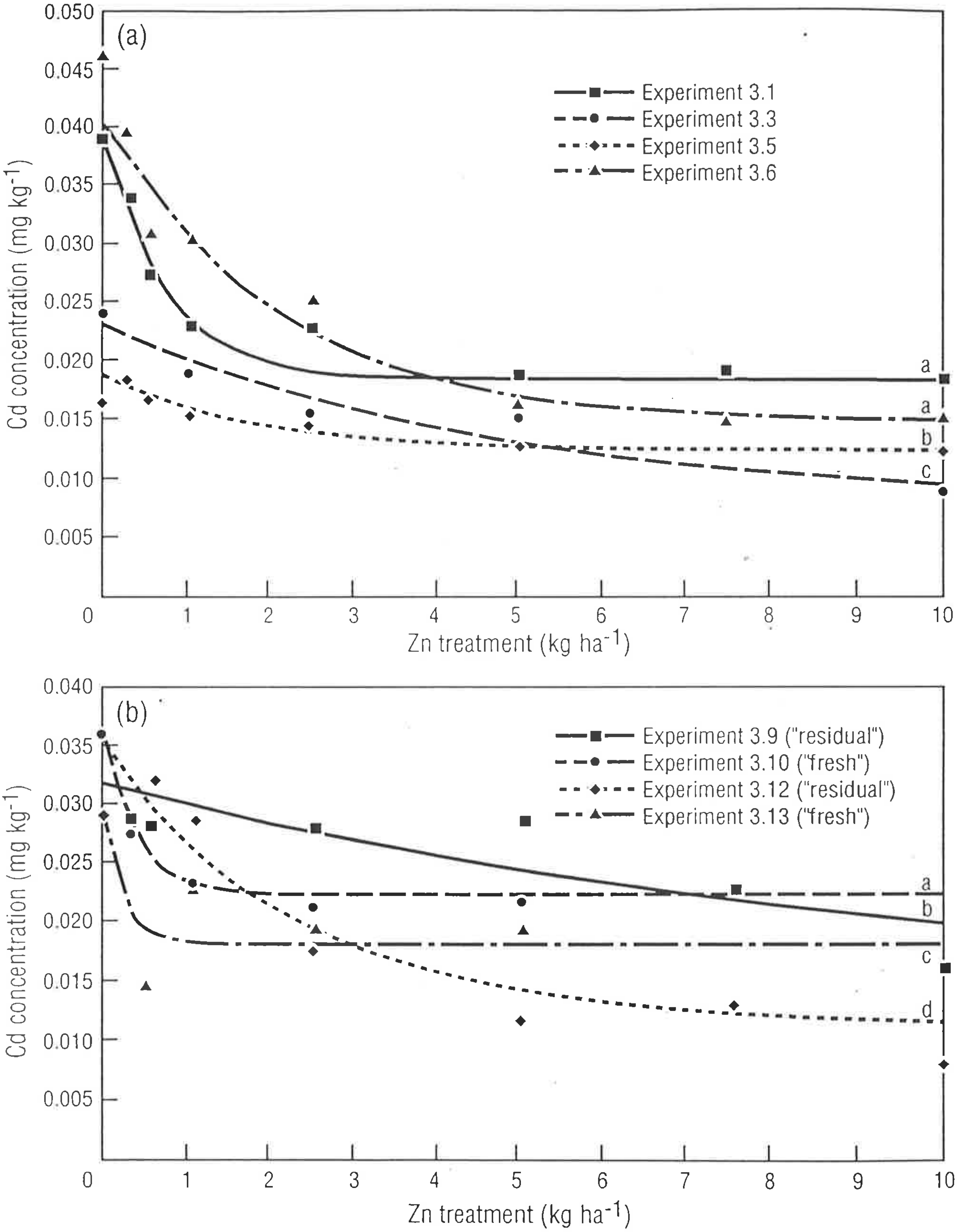

Figure 3.2. (a) and (b) Variation in grain Cd concentration (mg kg^{-1}) with Zn treatment (kg ha^{-1}) for (a) Experiments 3.1, 3.3, 3.5 and 3.6 and (b) Experiments 3.9, 3.10, 3.12 and 3.13. Exponential models were fitted to all the data in each experiment and are presented with the mean concentration for each Zn treatment. Statistical differences ($P<0.05$) between curves are distinguished by letters.

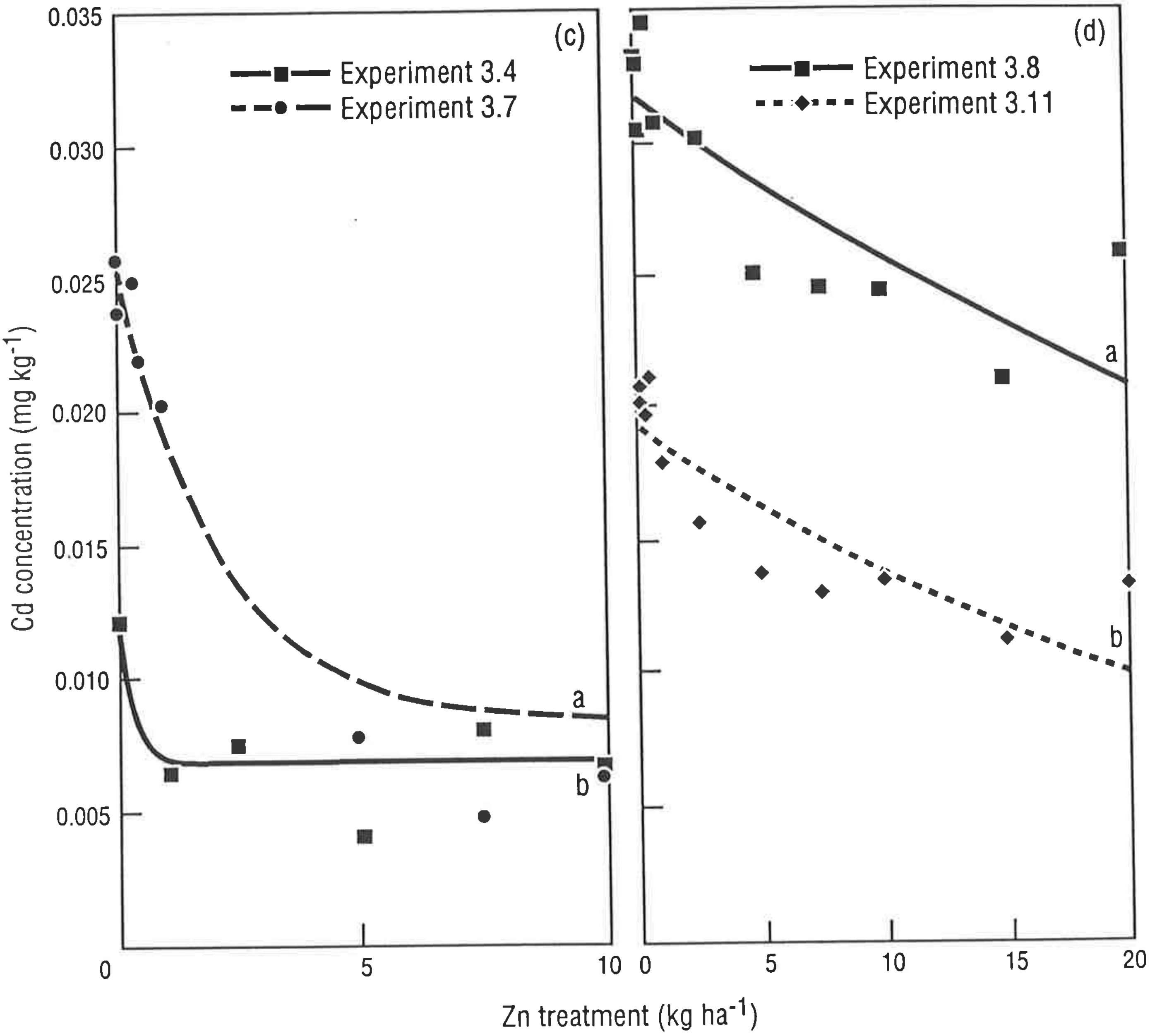

Figure 3.2. (c) and (d) Variation in grain Cd concentration (mg kg^{-1}) with Zn treatment (kg ha^{-1}) for (c) Experiments 3.4 and 3.7 and (d) Experiments 3.8 and 3.11. Exponential models are presented with the mean concentration for each Zn treatment. Statistical differences ($P<0.05$) between curves are distinguished by letters.

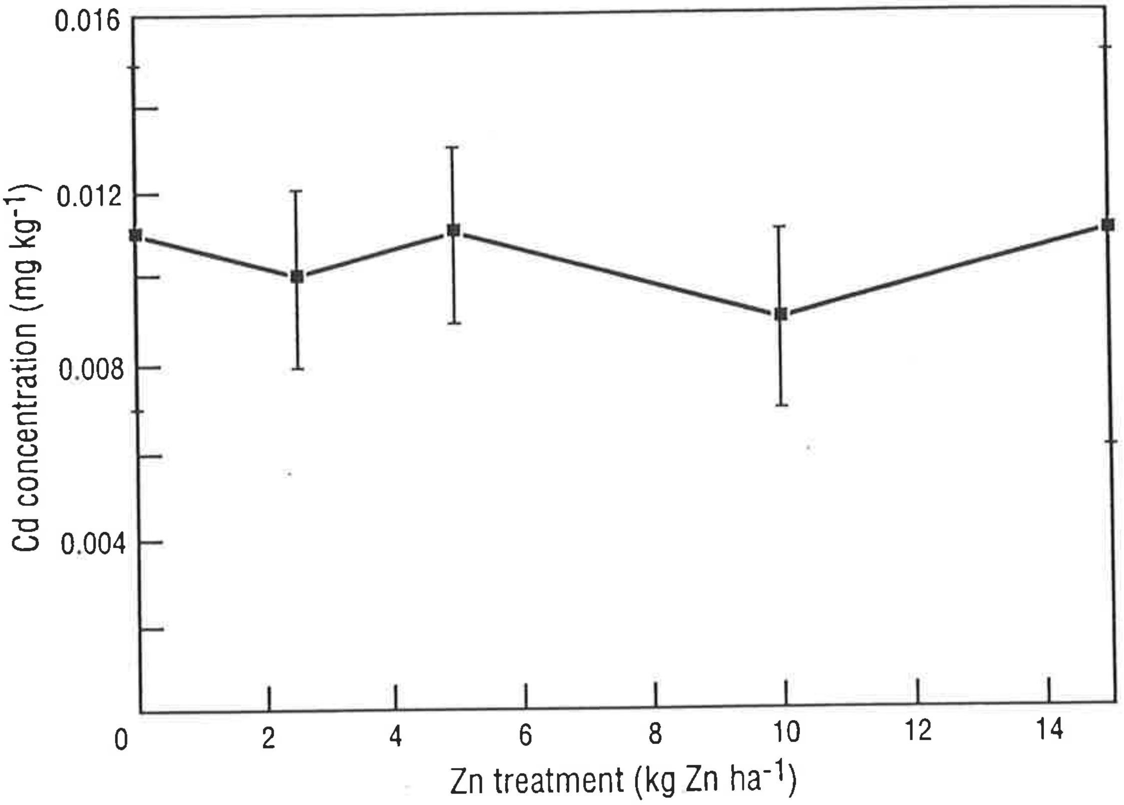

Figure 3.3. Variation in grain Cd concentration (±sd) (mg kg^{-1}) with Zn treatment for Experiment 3.17 (Kommamurra, Qld).

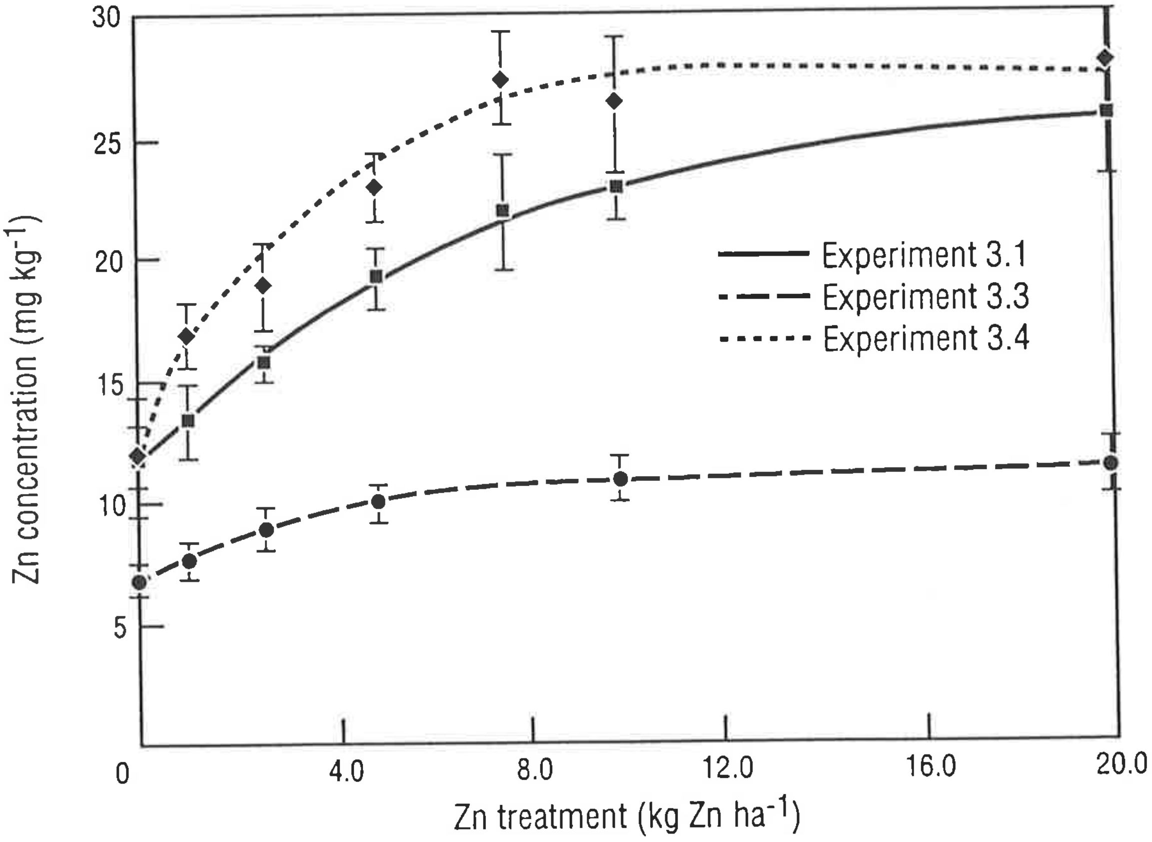

Figure 3.4 . Variation in mean grain Zn concentration (±sd) (mg kg^{-1}) with Zn treatment for Experiments 3.1, 3.3 and 3.4.

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Significant ($P<0.05$) grain yield responses following Zn applications were found in only six experiments in this study. In four experiments none of the decreased grain Cd concentration could be attributed to increases in grain yield following Zn applications. In the other two Zn responsive sites dilution due to increases in grain yield only partly contributed to the lower Cd concentrations.

The grain from the southern Queensland experiments had very low Cd (<0.01 mg kg^{-1}) concentrations near the detection limit which did not allow any trends with increasing rates of Zn to be seen. An example of the Cd concentration in grain is shown for Experiment 3.17 in Fig. 3.3.

3.3.2. Effect of "Residual" Zn on Cd Concentrations in Wheat Grain

Experiments at two sites (Ungarra and Yeelanna) on Eyre Peninsula permitted comparisons between the effect of "residual" Zn and "fresh" Zn applications on Cd concentrations in grain (Figs. 3.2b). Wheat grain from these experiments was harvested in the same growing season (1989) with the same variety (Machete) (Table 3.1). As with the other experiments, the Cd concentration in grain from both locations decreased as the Zn application increased. The exponential model was fitted to all data in each experiment and is presented with the mean concentrations for each Zn rate (up to 10 kg Zn ha^{-1}) for Experiments 3.9, 3.10, 3.12 and 3.13. There were no significant differences in grain Cd concentrations between the "fresh" Zn and "residual" Zn at Ungarra (Experiments 3.9 and 3.10) or the "fresh" Zn and "residual" Zn at Yeelanna (Experiments 3.12 and 3.13).

There were no further decreases in grain Cd concentration at Zn rates greater than approximately 1-2 kg Zn ha^{-1} for the "fresh" Zn treatments at both sites (Experiments 3.10 and 3.13) (Fig. 3.2b). However, the concentrations in grain grown with "residual" Zn (Experiments 3.9 and 3.12) however continued to decrease upto approximately 5-15 kg Zn ha^{-1}. The ODCd$_{50}$ for Experiments 3.10 and 3.13 ("fresh"

Zn applications) occurred at approximately the same Zn rate (0.26 and 0.16 kg Zn ha^{-1}, respectively) (Table 3.4). By contrast, half the observed decrease in grain Cd concentration ($ODCd_{50}$) was found at higher Zn rates for Experiments 3.9 and 3.12 ("residual" Zn), i.e. 6.93 and 1.71 kg Zn ha^{-1} , respectively (Table 3.4).

3.3.3 Effect of Zn Fertilization on the Concentration of Elements other than Cd in Grain

The grain from only the South Australian experiments was analysed for elements other than Cd on the ICP. The grain was analysed for Al, Ca, Cu, Fe, K, Mg, Mn, Na, P, S and Zn. The only element which showed significant ($P < 0.001$) differences between Zn treatments for all experiments was Zn. With increasing rates of Zn applications, the concentration of Zn in the grain increased. An example of this effect is shown in Fig. 3.4 for Experiments 3.1, 3.3 and 3.4.

Highly significant ($P<0.001$) effects of Zn applications on the concentrations of other elements in the grain were also found as follows: Ca in Experiments 3.5, 3.12 and 3.13; Cu in Experiments 3.1, 3.3, 3.5, 3.7, 3.11 and 3.13; Fe in Experiments 3.6, 3.8 and 3.11; K in Experiments 3.11 and 3.12; Mg in Experiments 3.7 and 3.12; Mn in Experiments 3.1, 3.4, 3.7, 3.11 and 3.12; P in Experiments 3.7 and 3.12; and S in Experiments 3.1 and 3.3. Some of these elements showed a similar decrease in concentration in the grain with increasing application rates of Zn as was seen with Cd (Figs 3.2a - 3.2b). An example of this is shown for Cu concentration in grain from Experiments 3.11, 3.12 and 3.13 (Fig. 3.5).

TABLE 3.4 Exponential equations for the relationship between grain Cd concentration (mg kg^{-1}) and Zn rate (kg ha^{-1}) and adjusted R^2 values for each experiment. The equation is of the form : grain Cd concentration = a + b *exp (-k) Zn rate

Experiment	a	b	exp (-k)	Adjusted † R^2	Significance ‡	∇Zn rate for ODCd$_{50}$ (kg ha^{-1})
i "Fresh" Zn						
3.1	0.0184	0.0194	0.2590	0.46	***	0.51
3.3	0.0078	0.0151	0.8084	0.82	***	3.26
3.4	0.0068	0.0053	0.0317	0.38	***	0.20
3.5	0.0123	0.0064	0.5600	0.32	**	1.20
3.6	0.0147	0.0254	0.6178	0.72	***	1.44
3.7	0.0081	0.0174	0.6343	0.74	***	1.52
3.8	0	0.0318	0.9788	0.39	***	32.3
3.10	0.0223	0.0137	0.0670	0.44	**	0.26
3.11	0	0.0193	0.9668	0.52	***	20.5
3.13	0.0180	0.0104	0.0142	0.34	***	0.16
ii "Residual" Zn						
3.9	0.0130	0.0187	0.9048	0.51	***	6.93
3.12	0.1117	0.0213	0.6665	0.72	***	1.71

† adjusted R^2 value = 1-(residual mean square/total mean square)

‡ NS not significant
* P<0.05
** P<0.01
*** P<0.001

∇ Zn rate at which half the total observed decrease in Cd concentration in grain (ODCd$_{50}$) was found.

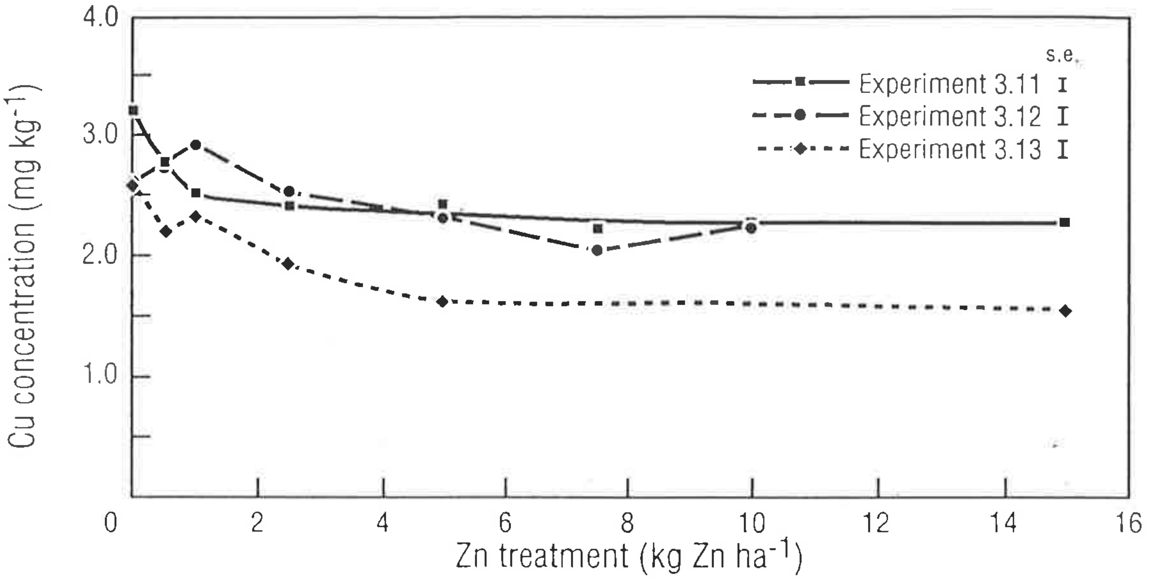


Figure 3.5. Variation in mean Cu concentration (mg kg^{-1}) in wheat grain with Zn treatments for Experiments 3.11, 3.12 and 3.13.

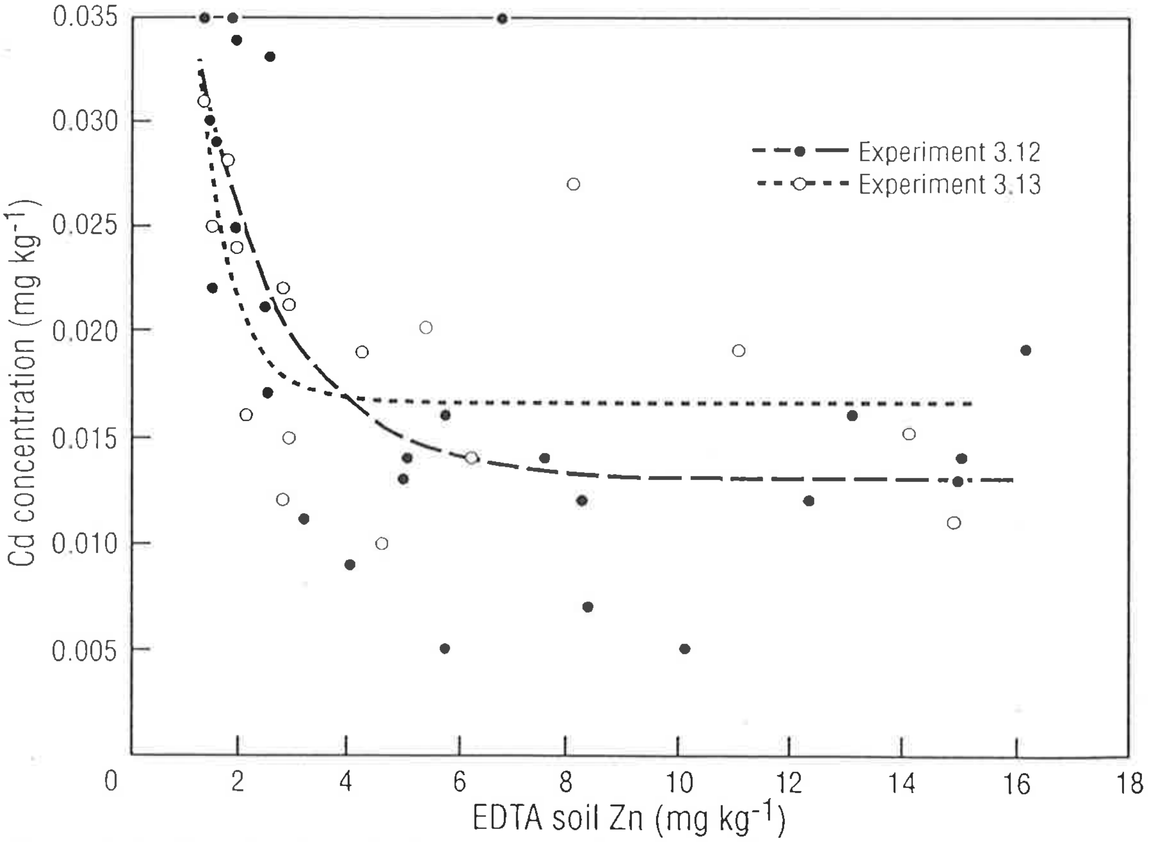


Figure 3.6. Variation in grain Cd concentration (mg kg^{-1}) with EDTA - extractable soil Zn (mg kg^{-1}) for Experiments 3.12 and 3.13.

3.3.4 Soil pH, EDTA-extractable Zn and EDTA-extractable Cd in the South Australian experiments.

Soil pH and EDTA-extractable Cd were determined to explain the variations in grain Cd concentration between sites. The range of mean soil pH across the Zn treatments of each South Australian experiment is given in Table 3.2b. For all experiments, except Experiments 3.4 and 3.5, there were no significant effects of Zn treatments on soil pH. Although the variation in soil pH for these two experiments was statistically significant the variation was small. For example in Experiment 3.4 the mean pH across Zn treatments ranged from 7.93 to 8.06. In Experiment 3.5, with the exception of one Zn treatment (2.5 kg ha^{-1}), the mean soil pH varied by only 0.3 pH units, ranging from 5.95 to 6.24 (Table 3.2b). The mean soil pH for Zn treatment 2.5 kg ha^{-1} in Experiment 3.5 was 5.69.

The EDTA-extractable Zn and Cd concentrations in soil for the control treatments are given in Table 3.2b. EDTA-extractable Zn fell within a concentration range broadly indicative of Zn deficiency in crops, i.e. < 2.0 mg Zn kg^{-1} (Merry and Tiller, unpublished data). EDTA-extractable Cd for all experiments varied from <0.01 - 0.08 mg kg^{-1} and were in the lower range of background values for EDTA-extractable Cd reported by Merry and Tiller (1991) for rural soils near Adelaide, South Australia. Further details of mean EDTA-extractable Cd concentrations for the South Australian experiments are given in Appendix 3.1.

3.4 DISCUSSION

3.4.1 Effect of Zn Applications on Cd Accumulation in Wheat Grain

The decrease in grain Cd concentrations with increasing Zn rates up to 2.5 - 5.0 kg Zn ha^{-1} (Figs. 3.2a - 3.2b) is in agreement with the findings of Honma and Hirata (1978) and Abdel-Sabour *et al.* (1988). Both groups worked with plants grown under conditions of Zn deficiency and both found Zn applications decreased Cd concentrations in rice roots and shoots grown in solution culture (Honma and Hirata,

1978) and Swiss chard shoots and maize shoots grown in a glasshouse on a silt-loam (Abdel-Sabour *et al.*, 1988). The significance of the results of the above two studies is that the same trend of decreasing grain Cd concentrations with increasing Zn rates was observed in the present field experiments across a range of soil types and soil Cd concentrations.

The results of the present study do not allow conclusions to be drawn as to whether the limiting effect of Zn on Cd uptake by wheat grain is a process related to plant physiology or to the chemical behaviour of these elements in the soil. Several processes may be involved.

Cadmium concentration in grain may decrease with increasing Zn applications due to direct competition between Zn and Cd for uptake by plant roots (Robson and Pitman, 1983). It has been hypothesised that Cd is able to compete successfully with Zn for the same active sites in animals but that it does not functionally substitute for Zn (Vallee and Ulmer, 1972). Although Vallee and Ulmer (1972) are referring to a biochemical substitution, Zn and Cd could possibly compete for uptake sites on plant roots. Smeyers-Verbeke *et al.* (1978) found that a fraction of the Cd absorbed by roots from solution involved metabolically mediated processes which were inhibited by Cu and Zn. Jarvis *et al.* (1976) observed a similar depression in short-term uptake of Cd by living roots of perennial ryegrass due to competition with Ca, Mn and Zn ions when grown in solution culture. Competition between Zn and Cd could also occur at other sites within the plant resulting in the decreased Cd concentration in grain with increasing concentrations of Zn.

Alternatively, loss of membrane integrity in Zn-deficient root cells (Welch *et al.*, 1982; Cakmak and Marschner, 1988) may permit Cd in soil solution to enter these root cells non-selectively by mass flow along with water. Applications of Zn to the soil to prevent the deficient status and the loss of membrane integrity would decrease the amount of Cd accumulating in the plant. Thus, plants grown under Zn deficient conditions may accumulate more Cd than plants grown in the presence of sufficient Zn. Phosphorus has similarly been found to accumulate in plants that are Zn deficient (Welch *et al.*, 1982).

As a consequence of the loss of membrane integrity in roots when plants are Zn deficient, small variations in concentrations in labile Zn in the soil may result in significant changes in Cd concentrations in grain under conditions of deficiency or near deficiency of Zn. Such changes may well prejudice prediction of Cd in grain from general relationships involving soil chemical tests. Soil extraction tests measure the extractable Cd, however the proportion of the extractable Cd that is accumulated in the plant may be dependent on the Zn status of the plant and the subsequent integrity of the root membrane. Under conditions of Zn deficiency or marginal Zn deficiency substantially higher Cd concentrations may be found in the grain than would be predicted from the relationship between grain Cd concentration and extractable soil Cd.

A further possibility is the mobilisation of Cd by phytosiderophores which are released by cereal roots in response to nutrient deficiency (Crowley *et al.*, 1987). Under severe Zn deficiency, root exudation of low molecular weight organic solutes, such as amino acids, sugars and phenolic compounds has been found to increase (Cakmak and Marschner, 1988). Zhang *et al.* (1989) observed immediate release of Zn mobilising root exudates at the onset of Zn deficiency. They showed that phytosiderophores were released by wheat and barley roots under conditions of Fe deficiency and that root exudates of Zn and Fe deficient plants mobilise both Zn and Fe.

The enhanced Cd accumulation in grain when wheat is Zn deficient is most likely a combination of both a loss of root membrane integrity and exudation of phytosiderophores by plants grown under Zn deficient conditions. The observation that the rate of decrease in Cd concentration in grain declined in an exponential manner with increasing rates of Zn (Figs. 3.2a - 3.2d) and that other elements (e.g. Cu, Mn and Fe) (Fig. 3.5) also decreased with increasing applications of Zn suggests the involvement of mechanisms other than direct competition for uptake.

3.4.2 Effect of "Residual" versus "Fresh" Zn on Cd Accumulations in Wheat Grain

In Fig. 3.2b the effect of Zn added at sowing ("freshly" added) is compared with effect of Zn applied 1 to 3 years earlier ("residual"). Generally, the Cd concentration in grain decreased as the Zn application increased. However, the Zn rate at which $ODCd_{50}$ occurred was usually greater for "residual" Zn than "fresh" Zn (Table 3.4). The Zn rate required to achieve $ODCd_{50}$ varied appreciably between experiments and reflects a complex interaction of several factors. For example, the availability to wheat of applied Zn will vary with soil characteristics and with elapsed time since application.

The different rates of decrease in Cd concentration with "residual" and "fresh" Zn treatments may be due to the occlusion of Zn, applied several years prior to harvest (i.e. "residual"), inside goethite particles which would make it less accessible for plant uptake. Brummer *et al.* (1988) found Ni, Zn and Cd were initially rapidly adsorbed onto the external surface of goethite, followed by a much slower solid-state diffusion of metals from external to internal binding sites. The metals in turn became fixed at positions inside the goethite particles. This explanation is more acceptable for the red light clay of Experiments 3.12 and 3.13, than to the siliceous sand of Experiments 3.9 and 3.10.

Furthermore, Zn may have leached out of the rooting zone. Leaching of Zn would be most likely to occur on the sand which would have a lower capacity to adsorb trace metals onto the surface of soil particles than the red light clay (Table 3.2a). There is some evidence of leaching of Zn or decreased availability of Zn for plant uptake from the EDTA-Zn data for experiments where the soil was sampled in the year of Zn application ("fresh") and 2-3 years after the application ("residual"). In Experiments 3.8 ("fresh") and 3.9 ("residual") the mean ± sd EDTA-Zn for Zn (mg kg^{-1}) treatments 1.0, 5.0 and 15.0 kg ha^{-1} were 1.23 ± 0.06, 3.26 ± 0.26 and 8.91 ± 0.04 and 1.14 ± 0.04, 2.21 ± 0.36 and 5.25 ± 0.46, respectively. The EDTA-Zn was considerably lower when resampled 2-3 years after the Zn application.

In conclusion, both "fresh" and "residual" Zn were effective in decreasing grain Cd concentrations. The time period for which a Zn application can effectively decrease grain Cd concentration is dependent on numerous factors, such as pH, that control Zn availability for plant uptake. Further investigations are required to determine the frequency of application of Zn required to maintain the grain Cd concentration in a designated range.

3.4.3 Soil Factors

Relationship between pH, EDTA-extractable Cd and Cd uptake

The mean EDTA-extractable Cd concentrations in soil (mg kg^{-1}) given for the control treatments in Experiments 3.1 and 3.3-3.13 (Table 3.2b) need to be considered in conjunction with the pH since EDTA-extractable Cd concentrations overestimate the soil Cd that is available for plant uptake (Symeonides and McRae, 1977). For example, although the mean EDTA-extractable Cd in the control of Experiment 3.7 is comparatively higher (0.044 mg kg^{-1}) than that in the control of Experiment 3.5 (0.011 mg kg^{-1}), the Cd concentration in grain from the control in Experiments 3.5 and 3.7 are almost the same. This is possibly due to a higher mean soil pH for the control of Experiment 3.7 (mean pH = 7.8) compared with those of Experiment 3.5 (pH = 5.97) (Table 3.2b). Therefore, although the soil at Lameroo (Experiment 3.7) has a higher EDTA-extractable Cd concentration than in Parilla (Experiment 3.5), it is likely that the Cd in the soil in Experiment 3.7 is less readily available for plant uptake due to the higher soil pH. Studies have shown that as the soil pH increases the Cd adsorbed to soil particles increases (Forbes *et al.*, 1976; Tiller *et al.*, 1984). The suitability of different soil extracts for predicting Cd concentration in grain is discussed further in Chapter 7.

Regressions of Cd concentration against EDTA-extractable Zn

Cadmium concentration in grain was plotted against EDTA-extractable Zn concentrations in the soil to take into consideration both the Zn concentrations in the soil prior to the application of Zn as well as Zn concentrations due to experimental treatments. An example is shown for Experiments 3.12 and 3.13 (Fig. 3.6).

These plots show that although the Cd concentration in grain decreased with increasing concentrations of EDTA-extractable Zn, the data were less scattered for Experiment 3.12 than Experiment 3.13. This is evident from the percentage variance accounted for by the exponential model: 56.1% and 39.1% for Experiments 3.12 and 3.13, respectively. In Experiment 3.12, the Zn treatments had been applied two years prior to the wheat crop, while in Experiment 3.13 the Zn treatments were applied in the same year as the crop was harvested (Table 3.1). Thus the wider scatter of data in Experiment 3.13 is most likely due to the Zn treatments being unevenly mixed through the soil surface layers. With the passage of time, and hence more cultivations, the Zn treatments in Experiment 3.12 would be more evenly distributed through the surface layers and consequently the availability of Zn to plant roots would be more uniform. Gartrell (1980) similarly showed that the effectiveness of Cu in granulated superphosphate in field experiments was greater in seasons subsequent to the season of application. He suggested this may be partly due to the disaggregation and dispersion through the soil of insoluble Cu phosphates by cultivation and pedoturbation.

Queensland Experiments (Experiments 3.14 - 3.17)

Experiments 3.14, 3.16 and 3.17 were on dark cracking clays while Experiment 3.15 was on a hard setting loamy soil with a mottled yellow clayey subsoil (Isbell *et al.*, 1967). The very low concentrations of Cd in grain from the Toowoomba region most likely reflect a combination of relatively high soil pH_{Ca}, ranging from 6.54 in Experiment 3.16 to 7.65 in Experiments 3.14 and 3.15 (Table 3.3), and high clay content (39% - 67%). The EDTA-extractable soil Cd concentrations of the two soils analysed were low (Table 3.3), suggesting Cd was strongly bound to these clays and hence less readily available for plant uptake.

3.4.4 Site Differences in the South Australian Experiments

Although the experiments were not designed to evaluate the effect of soil characteristics on Cd concentration in grain, the South Australian sites exhibited a great range of some variables that affect Cd availability, such as clay fraction (0 - 38%), organic carbon content (0.06 - 2.20%), pH_{Ca} (4.9-7.9) and EDTA - extractable Cd (0.011 - 0.083 mg kg^{-1}) (Table 3.2a and 3.2b). The relationship between grain Cd concentration (from 5 kg Zn ha^{-1} treatment) and these soil factors was determined by regression analysis. Data from the 5 kg Zn ha^{-1} treatment was used because generally at this Zn rate the Cd concentrations in grain had ceased to decrease any further. Mean grain Cd concentration decreased for each experiment when plotted against mean pH and was strongly correlated with pH ($r^2 = 0.50$, n=12). Eriksson (1990) similarly found a negative relationship between Cd concentration in 124 oat grain samples and soil pH ($r^2 = 0.24$) in a Swedish survey. Although clay content may be an important variable determining plant availability of Cd a plot of grain Cd against the clay fraction gave a poor correlation ($r^2 = 0.05$, n=12). Others have found that certain clay minerals, particularly oxides of Fe and Mn, exhibit highly specific adsorption affinity for Cd (Forbes *et al.*, 1976; Tiller *et al.*, 1984) and Zn (Shuman, 1975; Tiller *et al.*, 1984). The poor correlation between grain Cd concentration and clay content in this study is possibly due to the relatively narrow range of clay content in the soils since only two sites out of the nine soils had a clay content greater than or equal to 30%.

3.5 CONCLUSION

In most of the South Australian experiments on soils with low Zn concentrations the Cd concentration in grain decreased with increasing Zn rates up to 2.5 - 5.0 kg Zn ha^{-1} applied in the year of crop growth. This is in agreement with other studies of Cd uptake under conditions of Zn deficiency in solution culture (Honma and Hirata, 1978) and in soils in the glasshouse (Abdel-Sabour *et al.*, 1988). Generally, Zn applications were found to have a significant residual effect with Cd concentrations in grain still decreasing several years after the initial Zn application. The lack of response of Cd concentrations in grain to applied Zn in all Queensland experiments was most likely due to both low soil Cd concentrations, low availability of Cd in soils of high clay content and neutral soil pH values.

These results obtained from field experiments suggest that amelioration of Zn deficiency can have beneficial effects on wheat grain quality, by minimising Cd accumulation, as well as addressing the plants nutritional requirements for Zn.

CHAPTER 4

THE EFFECTS OF CROP ROTATION, STUBBLE MANAGEMENT AND TILLAGE PRACTICES ON CADMIUM CONCENTRATION IN GRAIN.

4.1 INTRODUCTION

In Australia, soil erosion and the decline in productivity of agricultural soils, particularly in marginal areas, have led to changes in farming practices. The rotation of crops such as lupins, beans, peas and other cereals with wheat to minimise disease, increase weed control and fix nitrogen, is a common management practice, with recent emphasis being placed on the inclusion of grain legumes in the rotation (Pratley and Rowell, 1987). To minimise soil erosion, stubble management practices have changed, with many farmers now retaining stubble on the surface of the soil rather than burning it (Callaghan and Millington, 1956). Changes in tillage practices and crop rotations have been reported to alter water infiltration, penetration of root systems and plant growth (Hamblin and Tennant, 1979; Gates *et al.*, 1981). Chemical changes have also been reported such as increased acidification (Nyatsanga and Pierre, 1973) and changes in the organic carbon content and cation exchange capacity of soils (McFarland *et al.*, 1990; Unger and Fulton, 1990; Smettem *et al.*, 1992). These physical, chemical and associated biological changes in the soil may affect the uptake of Cd by wheat.

This study was designed to investigate the effects of, and interactions between, modern crop rotations, stubble management and tillage methods on Cd concentrations in wheat grain.

4.2 MATERIALS AND METHODS

Two field experiments that had already been established in South Australia were sampled for this study. Selected physical and chemical characteristics and locations of these experiments are given in Table 4.1.

Table 4.1 Selected soil characteristics of the experimental sites

Experiment	Location	Classification[1]	Soil Texture	% Clay	% Silt	% Sand	pH_{Ca} range [2]	% Organic C range [2,4]	Range EDTA-extractable Cd (mg kg^{-1})[2]
4.1	Tarlee (34^0 16'S, 138^0 46'W)	Calcic Palexeralf	sandy loam	14	13	72	1986: 4.68-7.35 1987: 4.53-7.40	0.82 - 1.25[3]	0.08 - 0.20
4.2	Kapunda (34^0 21'S, 138^0 55'W)	Calcic Natrixeralf	sandy loam	11	21	69	4.65-5.10	1.3 - 1.9(*)	0.08 - 0.13

[1] Soil Survey Staff (1990)

[2] Range across plots in each experiment. All measurements have been made on 0-10 cm soil samples except those marked (*), which are 0-5 cm

[3] Mean values for 1986 and 1987

[4] Merry and Spouncer (1988)

4.2.1 Experimental Details

Experiment 4.1 : Tarlee rotation experiment

The rotation experiment at Tarlee was established in 1977 by the South Australian Department of Agriculture to monitor the effects of crop rotations and stubble management on soil properties and crop production (Schultz, 1988). The soil on which the experiment is conducted is a red brown earth (Stace *et al.*, 1968) or fine, mixed, thermic calcic palexeralf (Soil Survey Staff, 1990).

Wheat is grown every second year in two phases in each of eight rotations: continuous wheat, wheat/barley, wheat/peas, wheat/lupins, wheat/beans, wheat/volunteer pasture (variable composition but approximately 50% capeweed), wheat/sown pasture (almost 100% medic) and wheat/fallow. There are three stubble treatments : burning, incorporation into the soil and surface retention. Three rates of nitrogen (0, 40 and 80 kg ha^{-1}, termed N0, N40 and N80 in the text) as ammonium nitrate are applied to the wheat phase and to wheat and barley when grown as the alternate crop. Thus the wheat and barley plots received twice as much nitrogen as the other plots. The crop rotations and stubble treatments in Experiment 4.1 are completely randomised within two replicate blocks. The nitrogen treatments in Experiment 4.1 are randomised within each crop rotation - stubble treatment. The plots are 40 m long x 2 m wide and the treatments are replicated twice. Results of the agronomic effects of this experiment are presented elsewhere (Schultz, 1988).

Grain of wheat cv Warigal from all plots in 1986, except those treated with 40 kg N ha^{-1}, and from all plots in 1987 was analysed for Cd concentration as described in Chapter 3.

Experiment 4.2 : Kapunda rotation/tillage experiment

The experiment at Kapunda was established in 1983 by the CSIRO Division of Soils to study the effect of soil disturbance and crop rotation on soil-borne root diseases of wheat, mineral nitrogen concentrations, nitrogen fixation, soil structure, water use efficiency, and lupin and wheat yields. The results of studies on the effect of tillage and crop rotation on soil stability are presented elsewhere (Smettem *et al.*, 1992).

Wheat is grown every second year in each of three rotations : continuous wheat, wheat/volunteer pasture (which consists of approximately 50% sub clover and 40% grasses) and wheat/lupins. There are three tillage systems : direct drill, reduced till and conventional cultivation, and two fertilizer treatments : low - 15 kg P and 40 kg N ha^{-1} and high - 30 kg P and 80 kg N ha^{-1}. The crop rotations and tillage practices are completely randomised within three replicate blocks. Further details of the rotation and tillage treatments are given in Smettem *et al.* (1992). The phosphorus was applied as triple superphosphate and the nitrogen was applied as urea. The plots are 100 m x 2 m. The experiment is conducted on a fine, mixed, thermic calcic natrixeralf (Soil Survey Staff, 1990) or a red brown earth (Stace *et al.*, 1968).

Grain of wheatcv Spear from the 1989 and 1990 harvests was analysed for Cd content. In 1989, the alternate crop to wheat was sown while all rotations were sown to wheat in 1990.

4.2.2 Soil Measurements

Measurements of pH_{Ca} and EDTA-extractable Cd were made on soils from both Experiments 4.1 (Tarlee) and 4.2 (Kapunda) as described in Chapter 3. Since no soil was collected in the same year as the grain from Experiment 4.2 analysis was made on soil collected in 1991.

Calcium nitrate extractions

Soil (0-10 cm) from plots in the 1986 and 1987 harvests of Experiment 4.1 (Tarlee) from selected crop rotations (wheat/wheat, wheat/barley, wheat/lupins, and wheat/volunteer pasture) that had been treated with 0 or 80 kg N ha^{-1} was extracted with 0.01M $Ca(NO_3)_2$. Soil (0-5 cm) from all plots in Experiment 4.2 (Kapunda) were also extracted with 0.01M $Ca(NO_3)_2$.

The extraction involved shaking the soil samples for 4 hours with 0.01M $Ca(NO_3)_2$ in a 1:5 soil:solution ratio. Samples were then centrifuged at 4,000 rpm for 15 minutes. The soil:solution ratio and shaking time were determined from preliminary studies to give the best correlation between extracted Cd and Cd concentration in grain. An aliquot was drawn off and analysed with a graphite furnace atomic absorption (GFAA) spectrometer for Cd as detailed for Cd analysis of grain in Chapter 3. The Cd extracted with 0.01M $Ca(NO_3)_2$ was concentrated by solvent extraction using a procedure similar to that used to concentrate the Cd in acid digests of wheat as outlined in Chapter 3.

4.3 RESULTS

4.3.1 Cd Concentration in Grain

Cadmium concentrations in grain from Experiment 4.1 in 1986 and 1987 are shown graphically in Figs. 4.1 and 4.2 respectively. Analysis of variance showed significant ($P<0.001$) increases in Cd concentration in grain with increasing rates of nitrogen in both 1986 and 1987. The crop grown before the wheat crop had significant ($P<0.001$) effects on the Cd concentration in grain, with the highest Cd concentration being found in grain grown after lupins and the lowest Cd concentration in grain grown after barley. However stubble treatment had no significant effects on Cd concentrations in grain in either 1986 or 1987 (data not shown).

The coefficient of variation (cv) for the continuous wheat/N0 treatment in 1986 was very high (0.76). This was the result of extreme values for two treatments in replicate 1 : continuous wheat/stubble incorporated and continuous wheat/stubble retained. The mean grain Cd concentrations from duplicate determinations were 0.110 ± 0.024 and 0.156 ± 0.061 mg kg^{-1} for these two treatments, respectively. When the extreme values were removed the mean Cd concentration in grain for the wheat/N0 treatment became 0.038 ± 0.005 mg kg^{-1}. Repeating the analysis of variance with these extraneous values excluded resulted in stubble treatment also having highly significant ($P<0.001$) effects on the Cd concentration in grain from the 1986 harvest. Generally, higher Cd concentrations were found in grain from plots that had stubble incorporated than from plots that had stubble burnt or retained on the surface.

Significantly ($P<0.01$) higher Cd concentrations were found in Experiment 4.2 at Kapunda (1989) in wheat grain that had been direct drilled (Fig. 4.3). There was little difference in Cd concentration in wheat from the reduced till and conventionally cultivated plots of wheat from the continuous wheat rotation (Fig. 4.3). There were no significant effects of fertilizer treatments on grain Cd concentration.

In 1990, all rotations in Experiment 4.2 were sown to wheat (Fig. 4.4). Of the treatments, only crop rotation had significant effects ($P<0.001$) on the Cd concentration. Irrespective of cultivation practice, the highest mean Cd concentrations were found in grain grown after lupins, while for each cultivation practice there was little difference between mean grain Cd concentrations when grown after pasture or in a continuous wheat rotation (Fig. 4.4).

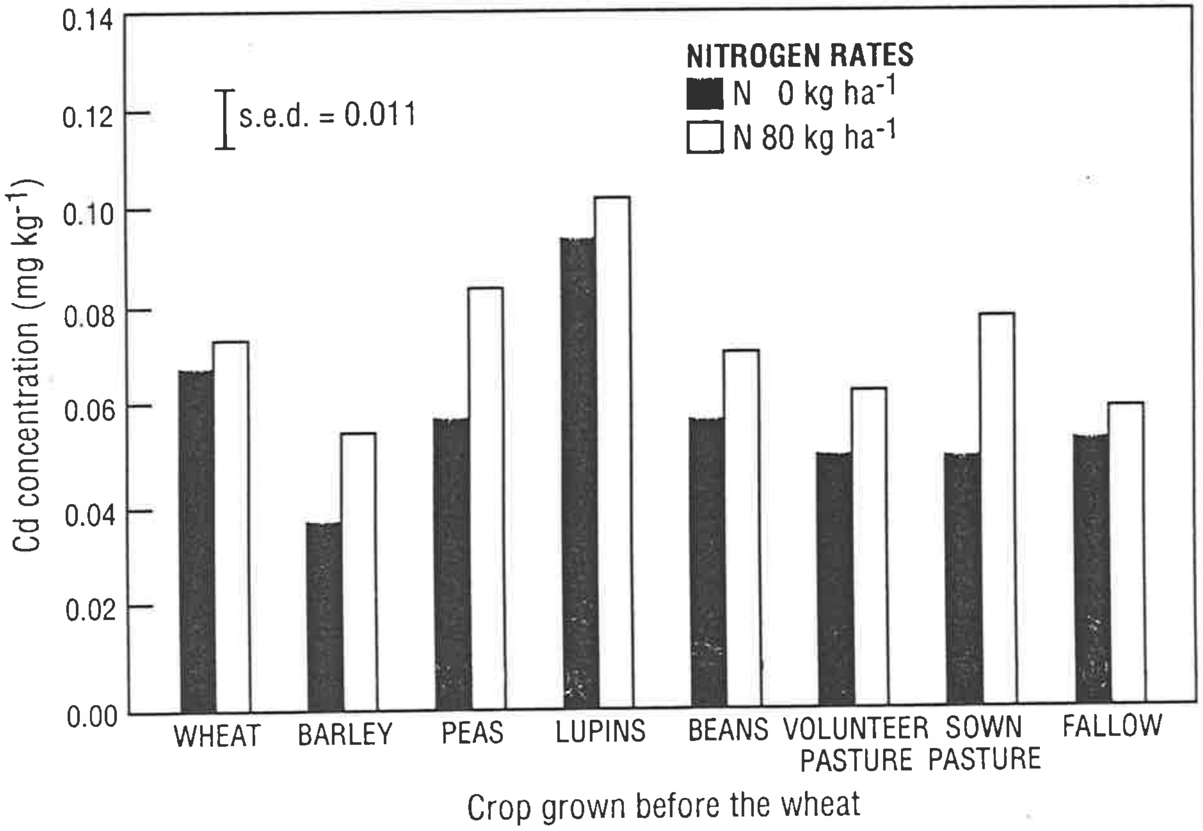


Figure 4.1. **Variation in grain Cd concentration in Experiment 4.1 at Tarlee (1986) for each rotation and two nitrogen rates (N0 and N80).**

Oliver, figure 1. D96, GER.

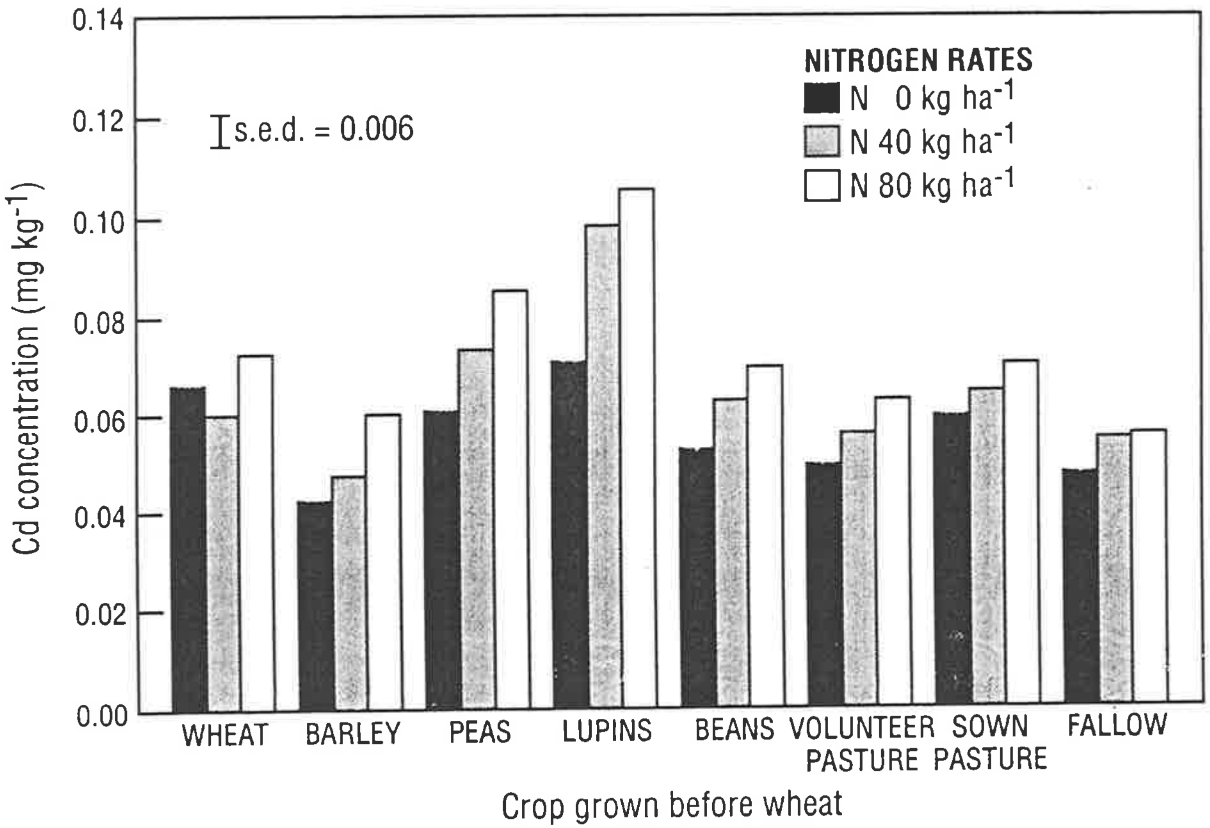


Figure 4.2. Variation in grain Cd concentration in Experiment 4.1 at Tarlee (1987) for each rotation and three nitrogen rates (N0, N40 and N80).

Oliver, figure 2. D96, GER.

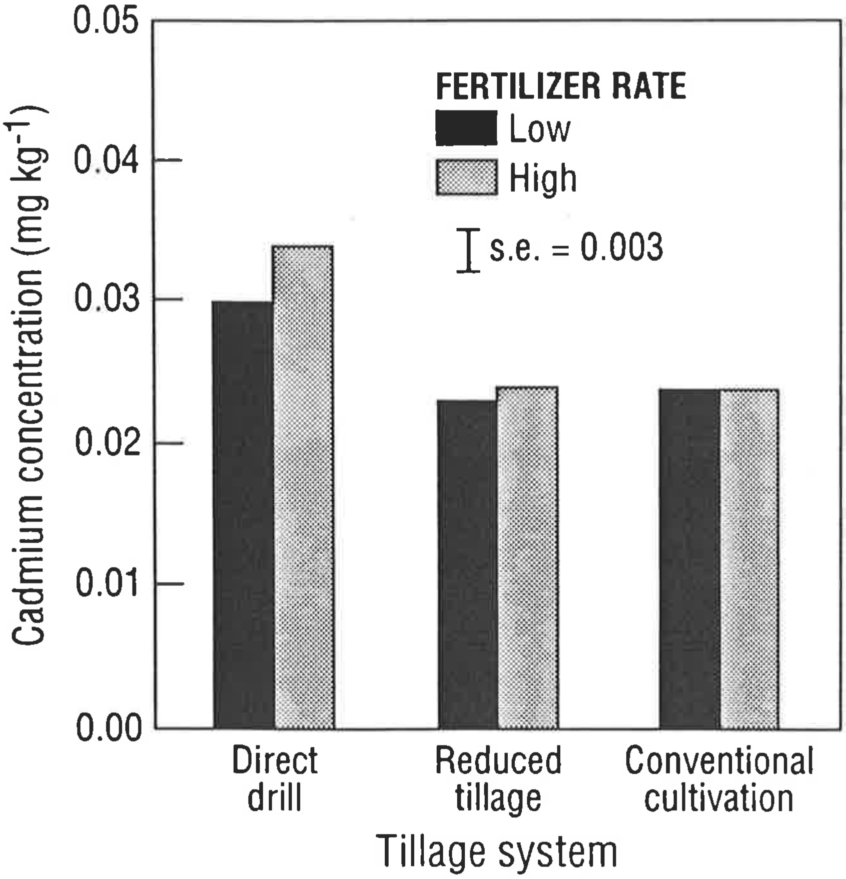


Figure 4.3. **Variation in Cd concentration in wheat grain grown in rotation with wheat for three tillage systems in Experiment 4.2 at Kapunda (1989).**

Oliver, figure 3. D96, GER.

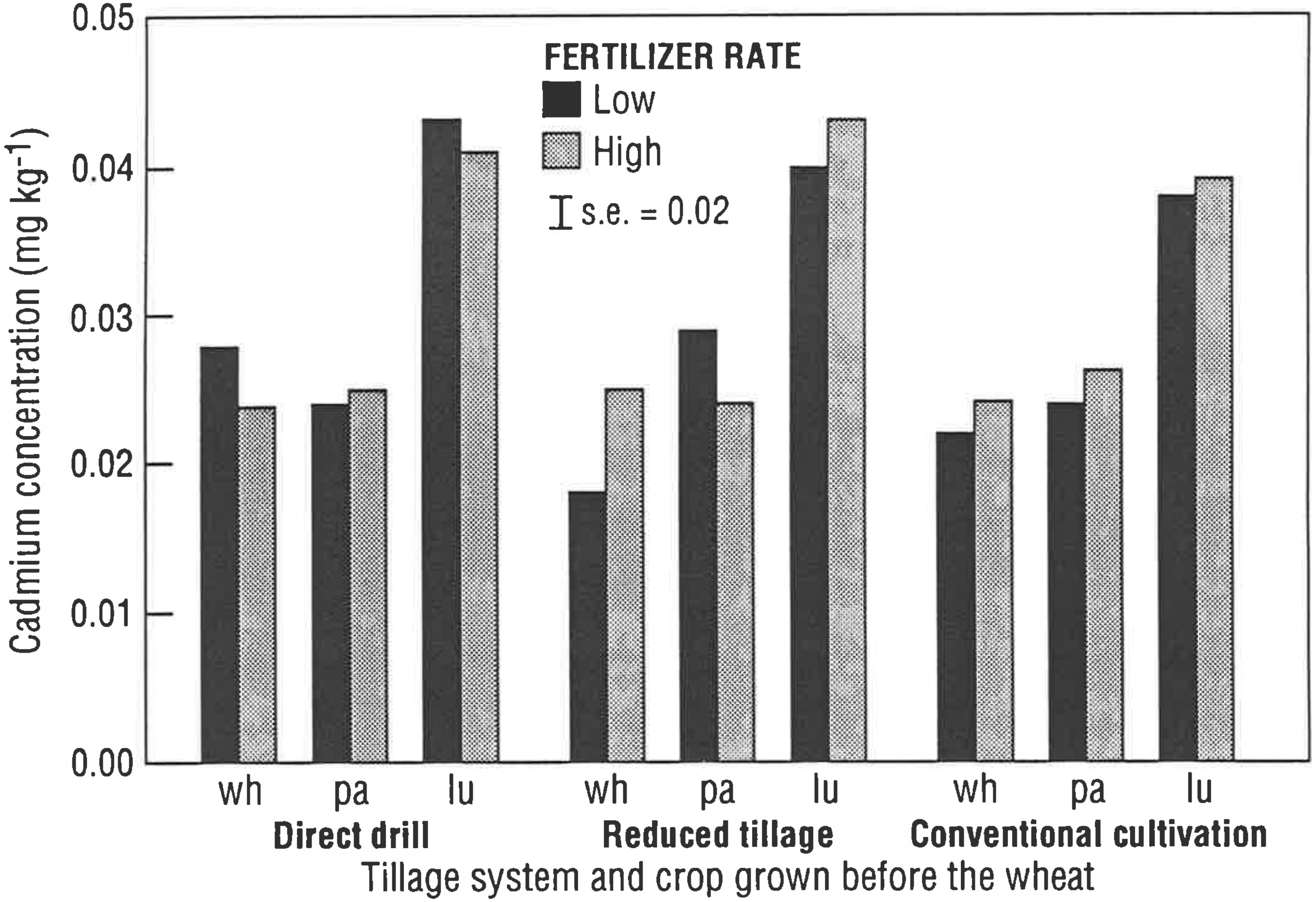


Figure 4.4. **Variation in grain Cd concentration in Experiment 4.2 at Kapunda (1990) in wheat grain grown in rotation with wheat (wh), pasture (pa) or lupins (lu) under three tillage systems. Low fertilizer: 15kg P ha^{-1} and 40kg N ha^{-1}; High fertilizer: 30kg P ha^{-1} and 80kg N ha^{-1}.**

Oliver, figure 4. D96, GER.

4.3.2 Soil Factors

Soil pH

In 1986, only crop rotation had significant effects ($P<0.05$) on soil pH in Experiment 4.1 (Tarlee). The mean pH_{Ca} values for samples collected at the 1986 harvest are shown in Table 4.2a and range from 5.29 for the wheat/bean - N80 rotation to 6.66 for the wheat/barley - N0 rotation.

Analysis of variance showed that both crop rotation and nitrogen treatments had significant ($P<0.05$) effects on soil pH_{Ca} in 1987. The mean pH_{Ca} values ranged from 4.80 for continuous wheat - N80 to 6.10 for the wheat/beans rotation - N0 (Table 4.2a). Stubble treatment had no significant effects in either year.

The mean pH_{Ca} values for the 1988 soil from Experiment 4.2 (Kapunda) ranged from 4.65 to 4.95 (Table 4.3a) and analysis of variance showed that only the effects of crop rotation were significant ($P<0.001$). The mean pH_{Ca} values for the three crop rotations were 4.71 (continuous wheat), 4.76 (wheat/pasture) and 4.89 (wheat/lupins). Although the highest soil pH_{Ca} was found in plots where lupins had been grown the previous year, the small difference in pH_{Ca} would not have had any major chemical effects on Cd availability for plant uptake.

EDTA- extractable Cd

In Experiment 4.1 only soils with the extreme nitrogen treatments (N0 and N80) were extracted with EDTA. Analysis of variance showed that crop rotation and crop rotation x nitrogen had significant effects ($P<0.05$) on EDTA-extractable Cd in 1986. The mean EDTA-extractable Cd concentrations ranged from 0.100 for the wheat/fallow - N80 treatment to 0.163 mg kg^{-1} for the wheat/beans - N0 treatment (Table 4.2b).

In 1987, the effects of crop rotation, nitrogen and stubble x nitrogen on EDTA-extractable Cd were significant, ($P<0.001$, $P<0.01$ and $P<0.05$, respectively). Further, the interaction effect of rotation x nitrogen just failed to achieve significance. The mean EDTA-extractable Cd concentrations for the rotation and nitrogen treatments ranged from 0.081 to 0.156 mg kg^{-1} (Table 4.2).

Soils collected from all plots of Experiment 4.2 in 1991 were extracted with EDTA (Table 4.3b). The only significant yet small effect was the interaction between cultivation and fertilizer rate ($P<0.05$). The highest mean EDTA-extractable Cd concentration was 0.110 mg kg^{-1} for the reduced tillage/high fertilizer rate treatment while the lowest was 0.092 mg kg^{-1} for the direct drill/high fertilizer treatment (Table 4.3b). The EDTA-extractable Cd concentrations across all plots varied by only 0.057 mg kg^{-1}, ranging from 0.074 to 0.131 mg kg^{-1} which is within the range expected to occur due to natural variability.

Calcium-nitrate extractable Cd

The $Ca(NO_3)_2$-extractable Cd in Experiment 4.1 (1986) was significantly ($P<0.05$) higher for soil from the wheat/lupins rotation than for the other rotations but the differences between rotations were not large. The range of mean ± s.d. $Ca(NO_3)_2$ -extractable Cd for each rotation in 1986 was: 5.4 ± 2.9, 4.4 ± 4.0, 8.3 ± 4.1 and 4.0 ± 3.3 µg kg^{-1} for the wheat/wheat, wheat/barley, wheat/lupins and wheat/natural pasture rotations, respectively. Stubble and nitrogen treatments had no significant effect on $Ca(NO_3)_2$-extractable Cd in 1986. However, in 1987 only the nitrogen treatment had a significant ($P<0.01$) effect on $Ca(NO_3)_2$-extractable Cd. Higher concentrations of $Ca(NO_3)_2$-extractable Cd were found in soil from plots that had been treated with the highest nitrogen treatment (N80) compared with soil from the plots that had not received nitrogen. The mean $Ca(NO_3)_2$-extractable Cd ± sd calculated across all rotations in 1987 were 5.5 ± 2.5 and 10.1 ± 5.7 µg kg^{-1} for N0 and N80, respectively.

The $Ca(NO_3)_2$-extractable Cd from soil in Experiment 4.2 (1990) was significantly ($P<0.001$) lower for the wheat/lupins rotation than for the continuous wheat and wheat/pasture rotations. The mean $Ca(NO_3)_2$-extractable Cd ± sd was 11.7 ± 1.5, 11.4 ± 2.1 and 7.2 ± 1.4 $\mu g\ kg^{-1}$ for the wheat/wheat, wheat/pasture and wheat/lupins rotations, respectively. Neither cultivation practices nor fertilizer rates had significant effects on the $Ca(NO_3)_2$-extractable Cd.

4.4 DISCUSSION

4.4.1 Soil pH and Cd Concentration in Grain

Generally as the soil pH increases the speciation of Cd ions and the charge on soil particles changes and the proportion of Cd in soil solution that becomes adsorbed onto the surfaces of soil particles increases as outlined in Section 2.2.1 Cadmium is bound to permanently charged surfaces on clay, to surface hydroxyl groups along the edges of clay particles, to Fe and Mn oxides and negatively charged sites on organic matter (Forbes *et al.* 1976). Consequently, any processes that acidify the soil might be expected to result in an increase in the Cd concentration in the grain.

Effects of nitrogen and phosphorus fertilizer

The application of ammonium nitrate as the nitrogenous fertilizer has been found to acidify the soil over a period of time (Helyar 1976; Bolan *et al.* 1991). Although a decrease in soil pH was generally seen with increasing rates of nitrogen in Experiment 4.1 (Table 4.2) the decrease was significant ($P<0.05$) only in 1987. Also, there were a few anomalies in the expected relationship between soil pH and Cd concentration in grain. For example, the wheat/beans rotation (1987) for both nitrogen treatments (N0 and N80) had the highest mean pH (Table 4.2) yet the grain from this rotation did not have the lowest Cd concentration (Fig. 4.2) as might be expected if soil pH was the main factor controlling Cd availability for plant uptake in these experiments.

Table 4.2 Mean soil pH (1:5, 0.01M $CaCl_2$) and EDTA-extractable Cd (mg kg^{-1}) for crop rotations and nitrogen treatments (NO and N80) of Experiment 4.1 in 1986 and 1987

(a)

	1986		1987	
Rotation	**NO**	**N80**	**NO**	**N80**
		pH		
continuous wheat	5.85	5.42	5.54	4.80
wheat/barley	6.66	6.04	5.78	5.36
wheat/peas	5.53	6.03	5.38	5.29
wheat/lupins	5.52	5.71	5.52	4.96
wheat/beans	5.36	5.29	6.10	5.94
wheat/natural pasture	6.03	5.96	5.69	5.27
wheat/sown pasture	6.34	5.69	5.50	5.43
wheat/fallow	6.26	5.49	5.51	5.92
L.S.D. (P=0.05)	0.82		0.65	

(b)

		EDTA-extractable Cd		
continuous wheat	0.120	0.138	0.114	0.121
wheat/barley	0.119	0.135	0.149	0.130
wheat/peas	0.131	0.134	0.145	0.145
wheat/lupins	0.118	0.138	0.151	0.132
wheat/beans	0.163	0.143	0.156	0.153
wheat/natural pasture	0.133	0.123	0.117	0.121
wheat/sown pasture	0.158	0.130	0.134	0.125
wheat/fallow	0.123	0.100	0.101	0.081
L.S.D. (P=0.05)	0.025		0.021	

Table 4-3a Mean soil pH (1:5, 0.01M $CaCl_2$) ± standard deviations for soil collected from Experiment 4.2 at Kapunda in 1988

Cultivation and Crop Rotation

Fertilizer Treatment	Direct Drill			Reduced Tillage			Continuous Cultivation		
	cont.wh	wh/pa	wh/lup	cont.wh	wh/pa	wh/lup	cont.wh	wh/pa	wh/lup
Low	4.73 ± 0.58	4.73 ± 0.15	4.90 ±0.00	4.80 ± 0.10	4.87 ± 0.21	4.90 ± 0.17	4.67 ± 0.12	4.70 ± 0.10	4.83 ± 0.06
High	4.70 ± 0.00	4.70 ± 0.14	4.95 ± 0.07	4.65 ± 0.07	4.80 ± 0.00	4.90 ± 0.14	4.70 ± 0.00	4.75 ± 0.07	4.85 ± 0.07

cont. wh. = continuous wheat
wh/pa = wheat/pasture
wh/lup = wheat/lupins

Table 4-3b Mean EDTA-extractable Cd concentrations (mg kg^{-1}) ± standard deviations for soils collected in 1991 at Experiment 4.2 (Kapunda)

Cultivation

Fertilizer Rate	Direct Drill	Reduced Tillage	Conventional Cultivation
Low	0.109 ±0.011	0.104 ±0.008	0.104 ±0.014
High	0.092 ±0.007	0.110 ±0.013	0.102 ±0.004

Despite the wheat and barley plots in Experiment 4.1 receiving twice as much nitrogen as the other plots these plots had one of the highest mean pH values in both years and the Cd concentration in wheat from the wheat/barley rotation was the lowest. One would expect that the Cd concentration in soil solution would increase due to the acidifying effect of nitrogenous fertilizer and consequently the concentration in wheat grain would increase.

Further there were no significant effects of fertilizer treatment on Cd concentration in grain in Experiment 4.2 (1989) (Figs. 4.3 and 4.4) despite the 'high' phosphorus and nitrogen treatment being twice the 'low' treatment. The expected effects of fertilizer treatment on Cd concentrations in grain were not seen possibly due to the low Cd concentration present as an impurity in the triple superphosphate. The effects of applying Cd in fertilizer on Cd concentration in wheat grain would be observed only after many years of fertilizer application.

Effects due to crop rotation

Acidification of the rhizosphere during legume growth may alter the speciation of Cd in the soil. Hence Cd may be more readily available for wheat grown in the following year than if the temporary acidification of the rhizosphere had not occurred. Legumes as well as other crops are known to acidify the rhizosphere and the extent of acidification is determined by the ratio of excess base to nitrogen in the crop (Nyatsanga and Pierre 1973). Smiley (1974) found a decrease of approximately 1 pH unit in the rhizosphere of cereals, peas and lupins when $(NH_4)_2SO_4$ was the N source compared with the pH of soil containing no plants. When $Ca(NO_3)_2$ was supplied as the N source the rhizosphere pH of cereals increased by 0.7 to 1.2 units while there was no change in the rhizosphere pH of lupins and peas compared with the pH of the soil containing no plants. Smiley (1974) also found the predominant form in which nitrogen is absorbed by roots strongly affected the rhizosphere pH. Generally the rhizosphere pH on container grown wheat plants was lower than the non-rhizosphere pH when NH_4 was supplied, higher when NO_3 was used and unchanged when both forms were used. After growing a lupin crop Cd may be transformed into a more weakly bound form and hence be more available for plant uptake. However, for the temporary change in pH to be effective the more weakly bound form of Cd must persist in the soil for several months and not be reversed when the pH rises again.

In both Experiments 4.1 and 4.2 the highest Cd concentration in grain was in wheat grown after lupins (Figs. 4.1, 4.2 and 4.4). The next highest mean Cd concentrations for both harvests of Experiment 4.1 were in grain from the wheat/peas, wheat/beans, continuous wheat or wheat/sown pasture rotations. The sown pasture consisted almost entirely of medic. Thus, apart from the continuous wheat rotation, the highest mean concentrations were found when the crops in the alternate phase to wheat were leguminous.

Linear regression analysis of Cd concentration in grain and soil pH showed a significant negative correlation for the wheat-lupin rotation in Experiment 4.1 (1987 harvest) ($R^2 = 0.63$, $P < 0.001$) and the wheat-pasture rotation in Experiment 4.2 (1990 harvest) ($R^2 = 0.31$, $P < 0.05$). There was no significant relationship between Cd concentration in grain and soil pH for any of the other rotations for either harvest of Experiment 4.1 or for the 1990 harvest of Experiment 4.2. (Details of the regression analysis are given in Appendix 4.1).

There were several anomalies in the relationship between pH and Cd concentration in grain from certain rotations, similar to the anomalies seen with the nitrogen fertilizer applications. For example in 1986 in Experiment 4.1, the highest Cd concentration was in grain from the wheat/lupin rotation - N80 (Fig. 4.1). However this treatment produced a higher mean soil pH value than that produced by the control nitrogen treatment for the same rotation (Table 4.2), for which a lower Cd concentration in grain would have been expected. Similarly in Experiment 4.2 (Kapunda) the highest mean pH was in soil from the wheat/lupins plots which produced grain with the highest Cd concentration (Fig. 4.3). This further suggests that factors other than just soil pH are involved in the increased Cd uptake into the grain of wheat by wheat grown after lupins.

4.4.2 EDTA-extractable Cd and Cd Concentration in Grain

Rotation and rotation x nitrogen had significant ($P<0.05$) effects on EDTA-extractable Cd extracted from the 1986 soils from Experiment 4.1. Although the highest Cd concentration was found in grain from the wheat/lupins rotation (Fig. 4.1), the corresponding soil had similar mean EDTA-extractable Cd concentrations to that from the wheat/barley rotation (Table 4.2) which had the lowest mean Cd concentration (Fig. 4.1).

EDTA is a strong extractant and provides a measure of the total potential available Cd present in the soil (Clayton and Tiller, 1979). Good correlations have been found between Cd concentrations in various plants and EDTA-extractable Cd when the plants were grown on soils with similar chemical characteristics. In this study EDTA-extractable Cd was not a good predictor of Cd concentrations in grain. This extractant is not sufficiently sensitive to detect small changes in Cd concentrations in wheat grain due to crop rotation. This was a conclusion shared by Symeonides and McRae (1977). However EDTA extractions do allow comparisons of soil Cd status to be made between sites. An extractant that is not buffered may provide a better correlation between Cd concentration in grain and the extracted Cd. For example, $Ca(NO_3)_2$ or some other extractant that does not complex Cd as strongly as EDTA, and hence gives a better indication of plant-available Cd may be more suitable. Symenoides and McRae (1977) found the best correlations between the concentrations of Cd extracted from the Cd-treated soils by various procedures and Cd concentrations in dry tops of the radish test crop, were with 1M NH_4NO_3. The effectiveness of EDTA, $Ca(NO_3)_2$

and $CaCl_2$ as soil tests to predict grain Cd concentration is discussed further in Chapter 7.

4.4.3 $Ca(NO_3)_2$-extractable Cd and Cd Concentration in Grain

Even though the differences in $Ca(NO_3)_2$-extractable Cd between rotations in Experiment 4.1 (Tarlee) (1986) were significant the differences were not appreciable. Correlations between extractable Cd and Cd concentration in grain were anomalous with $Ca(NO_3)_2$ as with EDTA. For example, although the highest grain Cd concentrations were found in wheat grown in rotation with lupins, significantly ($P<0.001$) lower $Ca(NO_3)_2$-extractable Cd was found in soil from the wheat/lupins rotation from Experiment 4.2 (Kapunda) and the effect of rotation on $Ca(NO_3)_2$-extractable Cd was not significant for the 1987 harvest of Experiment 4.1 (Tarlee). These anomalies may reflect problems with the sampling strategy rather than problems with the soil test. This matter is discussed further in Chapter 7.

4.4.4 Other Effects of Crop Rotation on Cd Uptake

Lupins have been found to produce exudates, such as citric acid (Nambiar 1976; Warembourg and Billes 1979; Gardner, 1981; Marschner 1991) which may bind Cd in a form that is more readily available to the crop grown in the following year. Soils associated with the roots of *Lupinus albus* have been found to contain more protons, reductants and chelating agents than the bulk soil. The main exudate from *L. albus* was citric acid and it has been hypothesised that citrate ions enhance uptake of metal ions such as Fe and Mn by lupins (Gardner 1981). Cadmium uptake may similarly be enhanced by lupin exudates. Again the altered form of Cd must persist in the soil for several months for lupin exudates to enhance uptake of Cd by the following wheat crop.

Another possibility is that certain crops may accumulate Cd to a greater concentration than other crops and when the stubble of those crops is returned to the soil the accumulated Cd is released during decomposition. Although in Experiment 4.2 (1989) lupins accumulated greater concentrations of Cd than wheat (Tiller *et al.*, 1993) detailed studies on the role of Cd released from different crop residues was not investigated. Further, if significant concentrations of Cd were being released from stubble decomposition one would have expected the stubble treatments to have had significant effects on Cd concentrations in grain, which was not the case in Experiment 4.1.

One method of testing the effects of roots and decomposition of roots on Cd uptake by wheat grain would be to grow both lupins and wheat in the glasshouse, harvest the shoot material and then determine the variation in the concentration of extractable Cd from soil around the roots with time.

4.4.5 Effects of Tillage

Grain from the continuous wheat rotation in Experiment 4.3 (1989) (Fig. 4.4) had the highest Cd concentration when direct drilled. Although there are no data on the effects of tillage practices on soil strength in these experiments, studies have been conducted on the effects of tillage practices on soil strength on similar soils.

Wheat grown on red-brown earths in the southern Australian wheat belt has generally shown a reduction in root growth under direct drilling compared with conventional cultivation (Hamblin and Tennant, 1979; Gates *et al.*, 1981). The major cause of restricted root growth was high soil strength in the surface 10 cm. After 13 weeks root growth had extended to the full depth of the soil profile (60-80 cm) but approximately 30% of the roots were in the top 10 cm (Hamblin and Tennant, 1979). Higher Cd concentrations may be expected in wheat when it is direct drilled because the restricted root growth would result in nutrients being taken up mainly from the surface soil horizons, where the highest soil Cd concentrations are located. This occurred in the 1989 harvest of Experiment 4.2 (Kapunda).

Tillage practices can also affect chemical properties of a soil. Smettem *et al.* (1992) found direct drilling at Experiment 4.2 (Kapunda) increased organic carbon and the total nitrogen in the soil. The highest Cd concentrations were found in direct drilled grain (Fig. 4.3) which may be due to increased mobility and hence availability for plant uptake of the organic matter-Cd complex.

4.5 SUMMARY AND CONCLUSIONS

At two field sites approximately 50 km apart Cd concentrations in grain were significantly higher in wheat grown after lupins compared with wheat grown after wheat. The lowest grain Cd concentrations in both experiments occurred when wheat was grown after barley. The effects of lupins grown in the previous year on Cd uptake by wheat cannot be explained in terms of soil pH and may involve other chemical or physical effects due to lupin growth.

The application of nitrogen fertilizer increased the grain Cd concentration irrespective of the crop rotation. Stubble management had no effect on Cd uptake by wheat and the effects of cultivation practice were generally significant only in the 1989 harvest of Experiment 4.2 (Kapunda). In the continuous wheat rotation the highest Cd concentrations were found in grain that was direct drilled compared with reduced till or conventional cultivation. This effect may be due to roots growing mainly in the upper soil horizons where the highest concentrations of Cd occur.

Results from this study suggest that growing wheat after lupins would result in higher Cd concentrations in grain than when wheat is grown in rotation with a cereal or pasture. This could have important implications for cereal growing in areas in Australia, particularly in Western Australia and the southern Eyre Peninsula, South Australia, where wheat is commonly grown in rotation with lupins. Further soils in these areas have a lower clay content than soils in some wheat growing regions on the east coast of Australia; the adsorptive capacity of these lighter-textured soils in southern Australia would be low and Cd may therefore be more readily available for plant uptake.

CHAPTER 5

EFFECTS OF SOIL pH ON CADMIUM CONCENTRATION IN WHEAT GRAIN GROWN IN A GLASSHOUSE

5.1 INTRODUCTION

Raising soil pH by liming is one of the most commonly recommended practices that a farmer can use to minimise Cd uptake by crops (Page *et al.*, 1981; Alloway, 1990). The influence of pH can be attributed to several factors including the effect of pH on ionic speciation and on the surface charge of mineral and organic soil fractions, which determine the availability of sites for adsorption and the activity of Cd in soil solution (Eriksson, 1989).

The effectiveness of raising soil pH to minimise Cd uptake by plants however has been quite variable. While some researchers (Andersson and Nilsson, 1974; Williams and David, 1976; Street *et al.*, 1978) found liming the soil decreased Cd uptake by plants, others have found no effect of liming on Cd uptake both in the glasshouse (McLean, 1976) and the field (Pepper *et al.*, 1983). Many investigators have used high concentrations of Cd applied as a salt or as sewage sludge, which cannot be directly related to Australian agricultural soils that have much lower soil Cd concentrations.

The purpose of this investigation was to study the interaction of soil type and Cd added to the soil on the effectiveness of raising soil pH to minimise Cd concentration in grain. The effects of raising soil pH to minimise Cd accumulation in grain were also studied in the field and details of this study are given in Chapter 6.

5.2 MATERIALS AND METHODS

5.2.1 Experimental Conditions

The investigation was conducted in a glasshouse with 200 mm wide x 200 mm deep pots. Four soils varying in their physical and chemical characteristics were used and details are given in Table 5.1.

Table 5.1 **Selected chemical and physical properties of the soils used in the glasshouse experiment.**

Parameter	**Inman Valley**	**Kapinnie**	**Freeling**	**Bordertown**
Classification[1]	Sodic Haploxerert	Mollic Palexeralf	Typic Rhodoxeralf (Sodic)	Natric Palexeralf
Particle size distribution:				
% sand (0.02-2mm)	26.0	52.8	78.9	86.9
% silt (0.002-0.02mm)	17.6	32.2	10.0	4.6
% clay (<0.002mm)	45.6	15.2	9.2	7.6
Soil texture	clay	sandy loam	loamy sand/ sandy loam	loamy sand
pH_{Ca} (1:5, 0.01M$CaCl_2$)	4.70	5.00	4.75	4.50
%C[2]	4.9	1.6	1.2	1.5
$NaHCO_3$-P[3] (mg kg^{-1})	38.5	58.5	55.3	52.2
$NaHCO_3$-K (mg kg^{-1})	340	232	400	190

[1] Soil Survey Staff, 1990

[2] Merry and Spouncer, 1988

[3] Colwell, 1963

The experiment had a four x five x four factorial design with four soils, five treatments to adjust pH and four amounts of Cd added as $CdCl_2.H_2O$. The treatments were replicated four times.

Sulphur (S) or calcium carbonate ($CaCO_3$) were added in amounts designed to give pH_{Ca} values of 4.0, 4.5, 5.0, 5.5 and 6.0. Details of the quantities of S or $CaCO_3$ added to each soil and the actual pH values obtained are given in Figure 5.4. The buffer curves used for each soil to determine the quantities of S or $CaCO_3$ required to reach the desired pH are given in Appendix 5.1.

An inoculum of S oxidising micro-organisms was added to each pot that had been treated with S. The inoculum was prepared in the following manner. One gram of S was added to 100 g of each soil which was moistened to a water potential of -10 kPa. The pH values of these mixtures were observed to decrease as the natural soil population of S oxidising micro-organisms began to produce acid. After about 3 weeks, distilled water was added to the cultures until there was sufficient inoculum to apply 100 mL per pot. The S and $CaCO_3$ added to adjust pH were allowed to equilibrate with the soil for 7 weeks before the Cd treatments were added.

The Cd treatments were added using a finely ground, acid-washed sand as the carrier medium. Different quantities of Cd were added to give a range of Cd concentrations in the soil. Details of the Cd analyses of the sand treatments and the quantities of sand mixed throughout each pot are given in Appendices 5.2 and 5.3, respectively. The quantities of sand mixed with each soil were such that the Cd applied to each soil was 0, 0.1, 0.3 and 0.9 mg kg^{-1} (called Cd 0, Cd 0.1, Cd 0.3 and Cd 0.9 in the text) in the Bordertown and Kapinnie soils, and 0, 0.3, 0.9 and 2.7 mg kg^{-1} (called Cd 0, Cd 0.3, Cd 0.9 and Cd 2.7 in the text) in the Freeling and Inman Valley soils. A higher Cd concentration (2.7 mg kg^{-1}) was added to the more heavily textured soils (Freeling and Inman Valley) rather than the 0.1 mg kg^{-1} because of concern that there would be little difference in grain Cd concentration between the 0 and 0.1 mg kg^{-1} treatments on these soils.

All pots were fertilised with 91 KH_2PO_4, 174 K_2SO_4, 171 $CaCl_2.2H_2O$, 24.6 $MgSO_4.7H_2O$, 10 $ZnSO_4.7H_2O$, 8.5 $MnSO_4.H_2O$, 0.5 $CoSO_4.7H_2O$, 0.8 H_3BO_3, 1.3 $CuSO_4.5H_2O$, 0.29 $(NH_4)_6Mo_7O_{24}.4H_2O$ and 93 mg kg^{-1} NH_4NO_3. An additional amount of KH_2PO_4 (38 mg kg^{-1}) was added to the Inman Valley soil because it had a lower $NaHCO_3$-extractable phosphorus content in comparison with the other soils (Table 5.1). All pots received a further 409 mg NH_4NO_3 approximately 8 weeks after sowing.

The Cd-treated soils were allowed to equilibrate by wetting and drying the soil 5 times over a period of several months. Six germinated seeds of wheat cv. Spear which were of uniform size, were sown in each pot on May 28, 1991; after the seedlings were well established the number of plants per pot was thinned to 3. Throughout the experiment deionised water was added 2 to 3 times per week to bring the water content in the soil upto 70% of water holding capacity (WHC). Moisture retention at 10 kPa suction was determined as a measure of WHC. The deionised water was checked for metal concentration every 2 months throughout the experiment. The electrical conductivity of the deionised water was compared fortnightly against that of the laboratory deionised water to ensure quality of glasshouse water.

All pots in each replicate block were randomly repositioned and the whole block of pots was moved on to the next bench every fortnight to minimise variation between replicates due to variation in environmental conditions within the glasshouse.

5.2.2 Harvest

After the plants were grown to maturity (approximately 32 weeks) the heads were collected separately from the other above-ground plant parts. The grain was manually threshed using a stainless steel thresher frame and a polyethylene grooved board. The foliar material was quickly washed in deionised water and then oven-dried at 60°C for 48 hours. The grain weight, grain number, total number of heads, total number of tillers and shoot dry weight from each pot were measured.

All the soil from the pots of the Kapinnie, Bordertown and Freeling soils was allowed to dry in the glasshouse and then sieved to pass through a 2 mm stainless steel mesh. Owing to the difficulties in removing the roots from the soil no root measurements or analyses were made. Sub-samples of sieved soil from each pot were dried at 40°C for measurement of pH_{Ca} and EDTA-extractable Cd as outlined in Chapter 3. $Ca(NO_3)_2$ extractions were made on two replicates of all soils as outlined in Chapter 4. Soil samples without added Cd for all pH treatments were also extracted with 0.01M $CaCl_2$ (1:5, soil:solution) using the same extraction and analytical procedure outlined for the $Ca(NO_3)_2$ extractions in Chapter 4. For both the $Ca(NO_3)_2$ and $CaCl_2$ extractions one third of the soil samples were analysed in duplicate on separate occasions.

Cadmium analysis of grain grown in soil to which no Cd was added was determined using the back-extraction procedure while a nitric acid digest was used for grain grown in soil to which Cd had been added as outlined in Chapter 3. The nitric acid digestion procedure was also used to analyse Cd concentrations in the shoot material.

5.3 RESULTS

5.3.1 Plant Growth and Yield

The effects of Cd treatments on the number of heads, number of tillers, plant dry weight and grain yield were generally not significant across all soil types. In those cases where the effects of Cd were statistically significant the actual differences were relatively small.

The S and $CaCO_3$ treatments had highly significant ($P<0.001$) effects on total numbers of heads and tillers produced by plants grown on all soils except Freeling (Fig. 5.1 and Appendix 5.4). Since the data for the total number of heads and number of tillers were similar only data for the former is presented. The total number of heads produced by plants grown on the Freeling soil increased significantly ($P<0.05$) at the highest rate of S. On the Inman Valley and Kapinnie soils the total heads produced per pot were also significantly ($P<0.001$) higher with increasing rates of S compared with the nil and lime treatments. By contrast on the Bordertown soil the total heads per pot generally increased significantly ($P<0.001$) at the highest rate of lime (Fig. 5.1).

In the text shoot dry weight does not include the weight of grain. Shoot dry weight increased significantly ($P<0.001$ and $P<0.01$) with the S treatment compared with the nil and $CaCO_3$ treatments for plants grown on the Inman Valley and Kapinnie soils, respectively. Again plants on the Bordertown soil showed the opposite effect with significantly ($P<0.001$) lower shoot dry weight with the S treatment and significantly ($P<0.001$) higher plant dry weight with the highest $CaCO_3$ treatment compared with the nil treatment. Although the treatments to alter pH also significantly ($P<0.05$) affected plant dry weight on the Freeling soil no trend was seen (Fig. 5.2).

Since the data for the mean number of grains produced per pot were similar to the data for mean grain weight per pot the former is presented in Appendix 5.5 and only data for grain weight is discussed. The mean weight of grain from Inman Valley soil increased significantly ($P<0.05$) with S treatments and with the lowest $CaCO_3$ treatment. No further increases in grain weight were found on the Inman Valley soil with increases in $CaCO_3$ rate. The weight of grain from plants grown on Bordertown, Kapinnie and Freeling soils decreased significantly ($P<0.001$) with S treatments and generally increased significantly ($P<0.001$) with increasing $CaCO_3$ rates (Fig. 5.3).

5.3.2 Cd Concentration in Wheat Grain

Cadmium, S and $CaCO_3$ treatments had highly significant effects ($P<0.001$) on Cd concentrations (mg kg^{-1}) in grain from plants grown on all four soils. However neither the S nor $CaCO_3$ treatments had any significant effect on Cd concentrations in grain when no Cd was added to the soil.

Grain Cd concentrations increased significantly ($P<0.001$) with increasing Cd additions to all soils. Cadmium concentrations in grain from plants grown on the Freeling soil generally increased significantly ($P<0.001$) with S treatments and decreased significantly ($P<0.001$) with the higher $CaCO_3$ rate compared with the control for the extreme Cd treatment (Cd 2.7) only (Table 5.2a). Grain Cd concentrations from plants grown on the Bordertown soil generally increased significantly ($P<0.001$) with the application of S and decreased as the rate of $CaCO_3$ appplication was increased to 0.95 g kg^{-1} soil (Table 5.2b).

Cadmium concentrations in grain from plants grown on the Inman Valley soil showed variable but negligible responses to the S and lime treatments depending on the Cd treatment. Grain Cd concentrations decreased with increasing $CaCO_3$ treatments for the Cd 0.9 treatment. Concentrations for the Cd 0, Cd 0.3 and Cd 2.7 treatments showed no statistical change with increasing rates of $CaCO_3$. For the Cd 0.9 and Cd 2.7 treatments the Cd concentration in grain decreased with the S treatment compared with the Cd concentration in grain grown in pots to which no S or lime had been added (Table 5.3a). The Cd concentrations in grain from plants grown on the Kapinnie soil increased significantly ($P<0.001$) with increasing rates of S but did not decrease significantly with increasing rates of lime compared with the control (Table 5.3b).

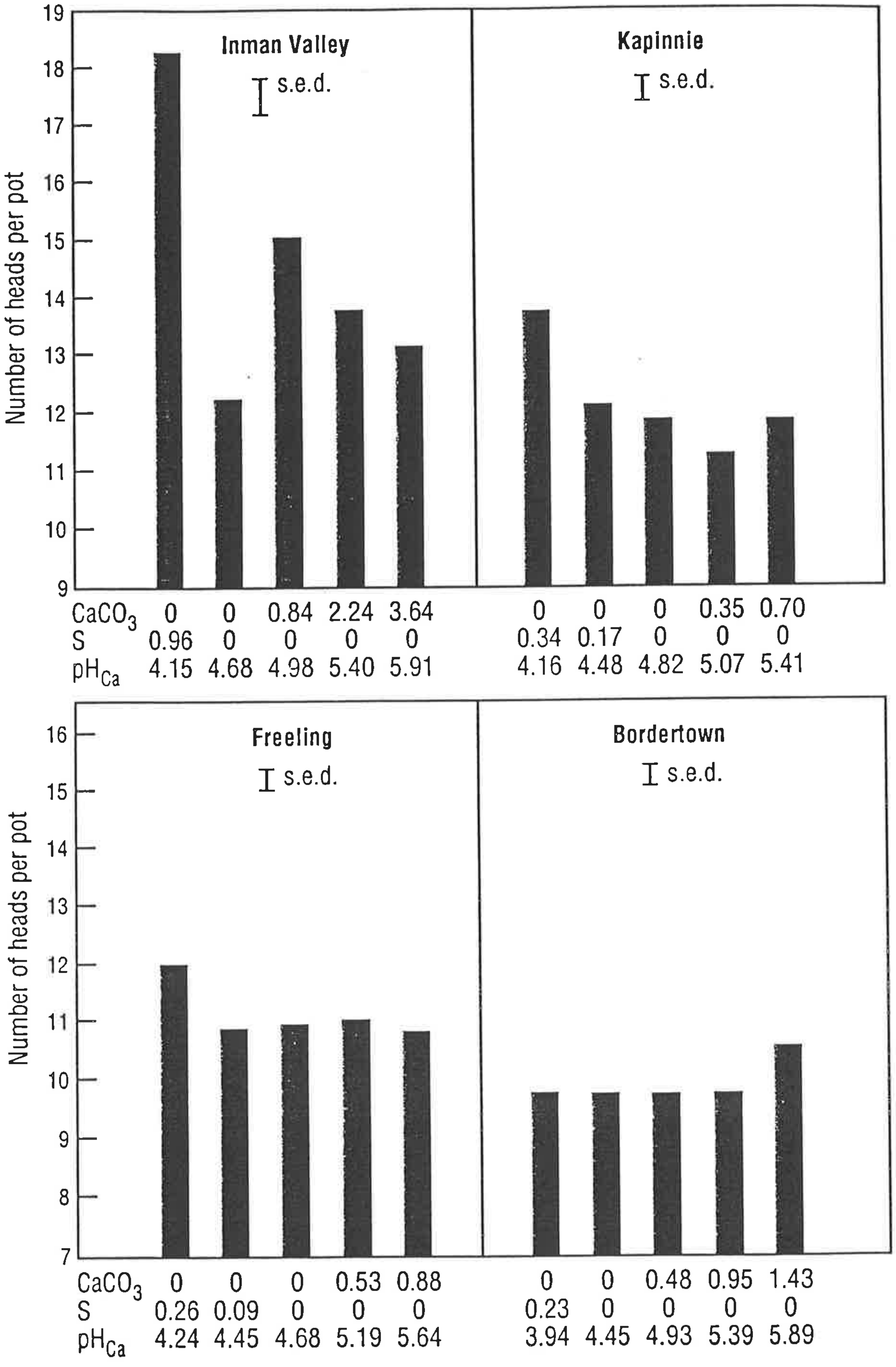


Figure 5.1. Mean number of heads per pot produced by plants grown in each sulphur (S) or calcium carbonate ($CaCO_3$) treatment (g kg^{-1}).

D. Oliver, figure 5.1. D100/GER.

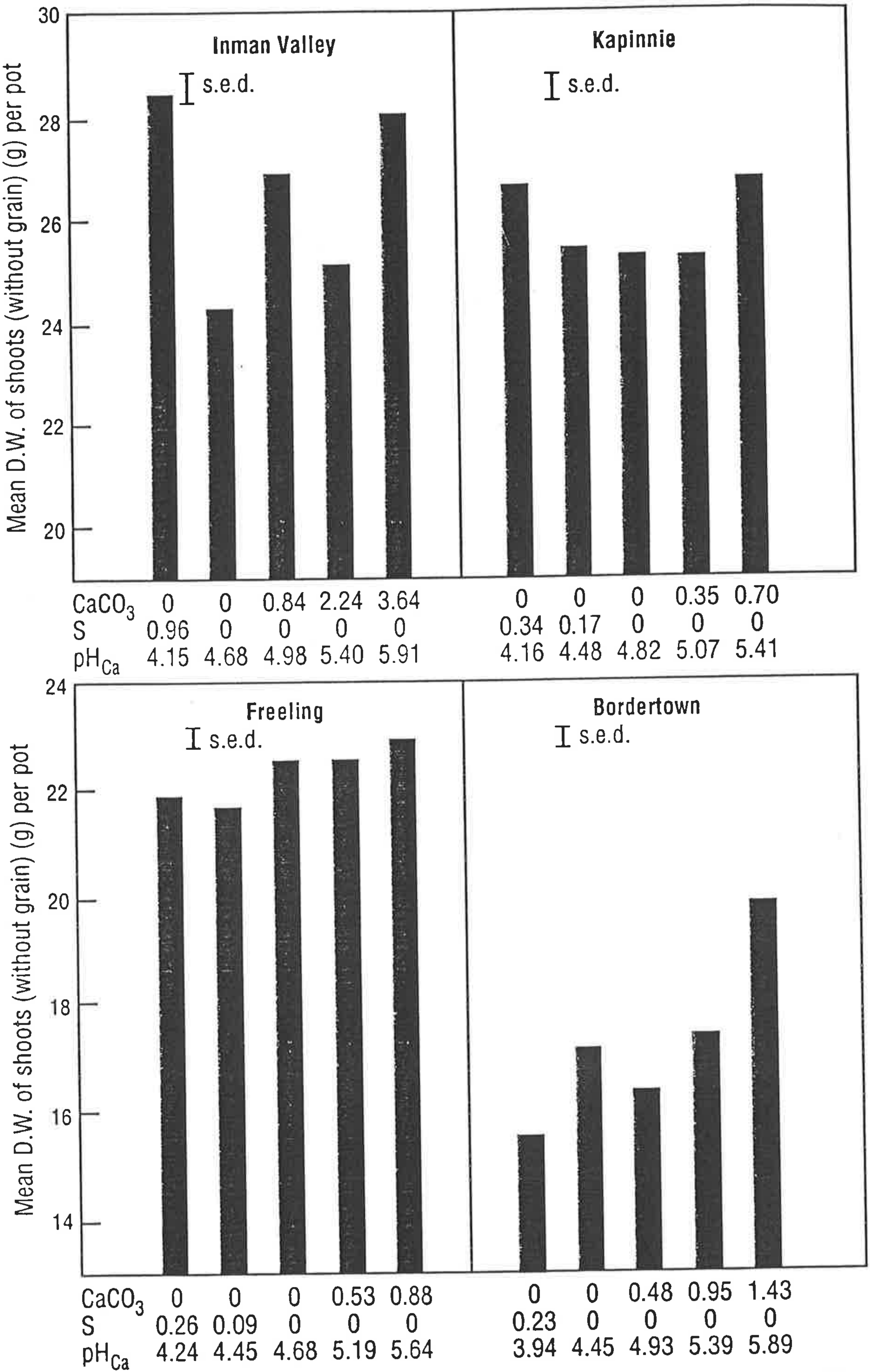


Figure 5.2. Mean dry weight (DW) of shoots (less grain) (g) per pot for each sulphur (S) or calcium carbonate ($CaCO_3$) treatment (g kg^{-1}).

D. Oliver, figure 5.2. D100/GER.

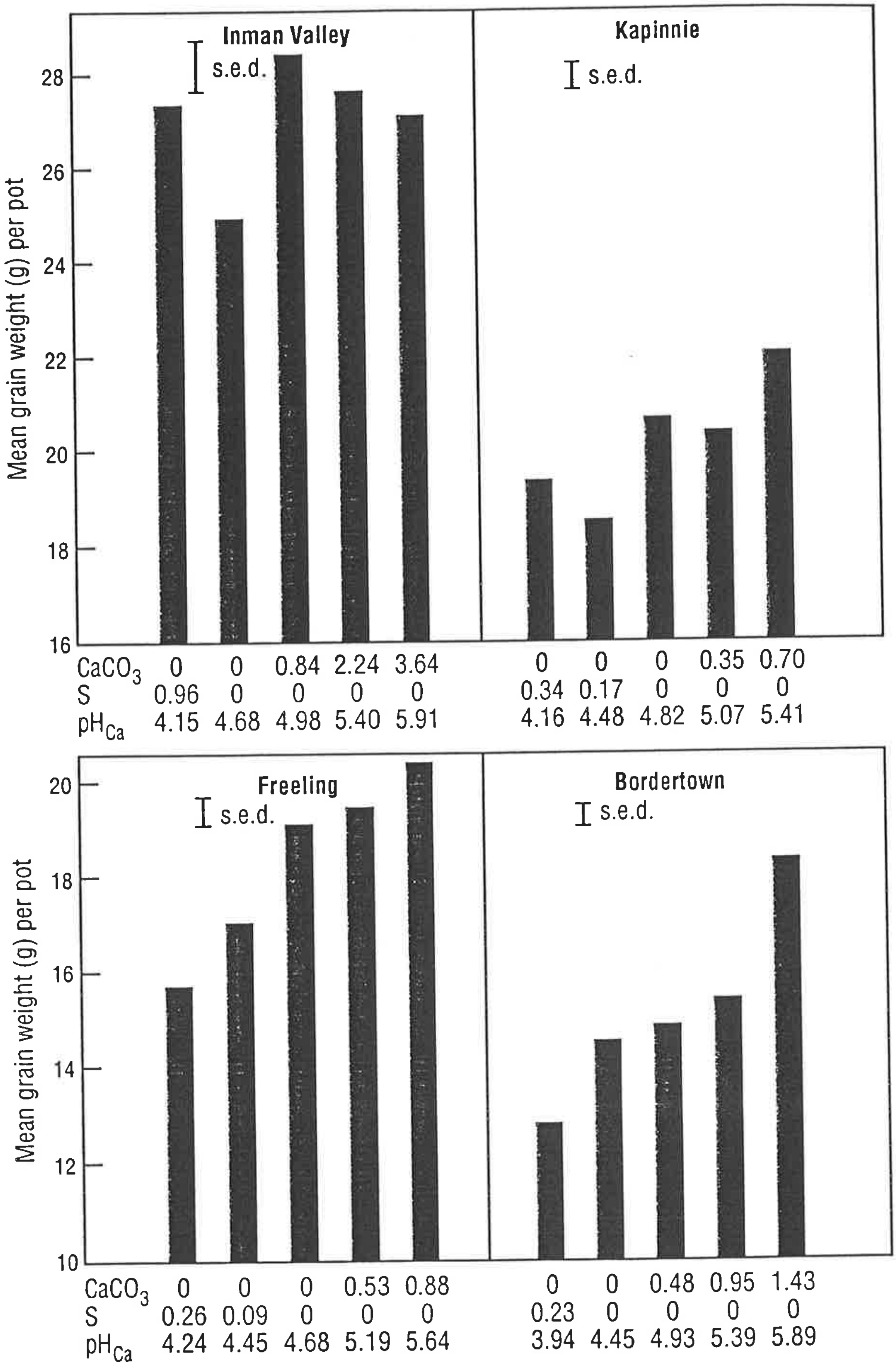


Figure 5.3. Mean grain weight (g) per pot for each sulphur (S) or calcium carbonate ($CaCO_3$) treatment (g kg^{-1}).

D. Oliver, figure 5.3. D100/GER.

Table 5.2 Mean grain Cd concentration (mg kg^{-1}) for each Cd and $CaCO_3$ or S treatment for plants grown in (a) the Freeling soil and (b) the Bordertown soil.

(a) Freeling

Cd treatment	g S kg^{-1}		Control	g $CaCO_3$ kg^{-1}	
	0.26	0.09	0	0.53	0.88
0	0.132	0.139	0.120	0.085	0.076
0.3	0.463	0.435	0.374	0.323	0.337
0.9	1.025	1.116	1.082	0.974	0.748
2.7	3.478	3.474	2.250	2.336	1.752

s.e.d. = 0.120

(b) Bordertown

Cd treatment	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
	0.23	0	0.48	0.95	1.43
0	0.174	0.162	0.121	0.088	0.067
0.1	0.438	0.286	0.263	0.170	0.147
0.3	0.541	0.451	0.454	0.275	0.292
0.9	1.280	0.984	0.812	0.587	0.497

s.e.d. = 0.051

Table 5.3 Mean grain Cd concentration (mg kg^{-1}) for each Cd and $CaCO_3$ or S treatment for plants grown in (a) the Inman Valley soil and (b) the Kapinnie soil.

(a) Inman Valley

Cd treatment	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
	0.96	0	0.84	2.24	3.64
0	0.075	0.088	0.113	0.079	0.068
0.3	0.165	0.185	0.182	0.193	0.162
0.9	0.344	0.585	0.475	0.395	0.363
2.7	1.006	1.283	1.549	1.319	1.403

s.e.d. = 0.054

(b) Kapinnie

Cd treatment	g S kg^{-1}		Control	g $CaCO_3$ kg^{-1}	
	0.34	0.17	0	0.35	0.70
0	0.213	0.175	0.108	0.096	0.104
0.1	0.311	0.238	0.116	0.161	0.157
0.3	0.335	0.384	0.238	0.223	0.318
0.9	1.367	0.880	0.634	0.618	0.583

s.e.d. = 0.050

5.3.3 Cd Content in Grain

Cadmium content in grain (μg pot^{-1}) was calculated by multiplying grain Cd concentration (μg kg^{-1}) by the weight of grain in each pot (kg pot^{-1}). Expressing the Cd content in grain on a per pot basis allowed the effect of yield to be assessed. As found with grain Cd concentration, Cd content of grain on all soils increased significantly ($P<0.001$) with increasing rates of Cd applied to the soil. Cd content on the Freeling and Bordertown soils increased significantly ($P<0.01$ and $P<0.001$, respectively) with S treatments and decreased with lime treatments at the highest Cd treatment (Cd 2.7) (Tables 5.4a and 5.4b). However, the changes in Cd content on the Bordertown soil for the other Cd treatments were negligible. While Cd content (μg pot^{-1}) in the Kapinnie soil also increased significantly ($P<0.001$) with increase in S treatments there was no change in Cd content with increasing lime treatments for the Cd 0, Cd 0.1 and Cd 0.9 treatments (Table 5.5b).

Cadmium content in grain on the Inman Valley soil was more variable for the four Cd treatments than seen on the other soils. There was little change in Cd content with S or lime treatments for the Cd 0 and Cd 0.3 treatments. There was no change in Cd content with the S treatments for the Cd 2.7 treatment but a significant ($P<0.01$) decrease for the Cd 0.9 treatment (Table 5.5a).

5.3.4 Cd Concentration in Shoots (excluding grain)

The shoot Cd concentration data was log-transformed to decrease the effect of large variability of high values. The log-transformed Cd concentration in the shoots on all soils increased significantly ($P<0.001$) with increasing concentrations of Cd added to the soil (Tables 5.6 - 5.7). Generally, the log-transformed Cd concentrations in the shoots increased significantly ($P<0.001$) with increasing rates of S and decreased significantly ($P<0.001$) with increasing rates of lime for plants grown on the Bordertown (Table 5.6b) and Kapinnie soils (Table 5.7b).

Table 5.4 Mean Cd uptake in grain (μg pot^{-1}) for each Cd and $CaCO_3$ or S treatment for plants grown in (a) the Freeling soil and (b) the Bordertown soil.

(a) Freeling

Cd treatment	g S kg^{-1}		Control	g $CaCO_3$ kg^{-1}	
	0.26	0.09	0	0.53	0.88
0	2	2	2	2	2
0.3	8	8	7	6	6
0.9	16	19	18	18	15
2.7	49	54	42	42	37

s.e.d. = 3

(b) Bordertown

Cd treatment	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
	0.23	0	0.48	0.95	1.43
0	2	2	2	2	1
0.1	6	4	4	3	3
0.3	7	7	7	4	5
0.9	15	14	12	9	10

s.e.d. = 0

Table 5.5 Mean Cd uptake in grain ($\mu g\ pot^{-1}$) for each Cd and $CaCO_3$ or S treatment for plants grown in (a) the Inman Valley soil and (b) the Kapinnie soil.

(a) Inman Valley

Cd treatment	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
	0.96	0	0.84	2.24	3.64
0	2	2	3	2	2
0.3	4	5	5	6	4
0.9	10	16	15	11	9
2.7	28	30	37	34	41

s.e.d. = 2

(b) Kapinnie

Cd treatment	g S kg^{-1}		Control	g $CaCO_3$ kg^{-1}	
	0.34	0.17	0	0.35	0.70
0	4	3	2	2	2
0.1	6	4	3	3	3
0.3	7	8	5	4	7
0.9	27	16	13	13	13

s.e.d. = 1

The log-transformed Cd concentration in shoots grown on the Inman Valley soil decreased with the S treatment for all Cd rates. There were no statistical differences between the log-transformed Cd concentrations in shoots in the Inman Valley soil at the highest lime and the S treatments at the Cd 0, Cd 0.3 and Cd 0.9 treatments (Table 5.7a).

Although the log-transformed Cd concentrations in shoots in the Freeling soil generally increased significantly ($P<0.001$) with increasing rates of lime there was no statistical difference between the concentrations for the control and the S treatment (Table 5.6a).

5.3.5 Soil Factors

pH and EDTA - extractable Cd

As expected, soil pH decreased significantly ($P<0.001$) with S treatments and increased significantly ($P<0.001$) with increasing lime treatments in all soils (Fig. 5.4). Cadmium treatments had no significant effect on soil pH and there was no significant interaction between treatments on pH in any soil.

Also as expected, EDTA-extractable Cd increased significantly ($P<0.001$) with increasing Cd rates in all soils (Fig. 5.5). Lime and S treatments had no significant effect on EDTA-extractable Cd. Despite equilibrating the Cd treatments in the soils for several months most of the applied Cd was extracted with EDTA (Fig. 5.5).

$Ca(NO_3)_2$ - and $CaCl_2$ - extractable Cd

Both Cd treatments and treatments to adjust soil pH had highly significant ($P<0.001$) effects on $Ca(NO_3)_2$-extractable Cd in all four soils. The $Ca(NO_3)_2$-Cd decreased significantly ($P<0.001$) with increasing rates of lime and increased significantly ($P<0.001$) with increasing rates of S. The $Ca(NO_3)_2$ concentrations also increased significantly ($P<0.001$) with increasing rates of Cd (Tables 5.8-5.9).

Table 5.6 Log_{10} mean Cd concentration (mg kg^{-1}) in shoot material for each Cd and $CaCO_3$ or S treatment for plants grown in the (a) Freeling and (b) Bordertown soils.

(a) Freeling

Cd treatment	g S kg^{-1}		Control	g $CaCO_3$ kg^{-1}	
	0.26	0.09	0	0.53	0.88
0	-0.246	-0.294	-0.369	-0.481	-0.555
0.3	0.293	0.283	0.200	0.220	0.070
0.9	0.768	0.743	0.687	0.611	0.507
2.7	1.177	1.255	1.235	1.230	1.103

s.e.d. = 0.092

(b) Bordertown

Cd treatment	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
	0.23	0	0.48	0.95	1.43
0	-0.182	-0.225	-0.434	-0.523	-0.723
0.1	0.195	0.073	-0.146	-0.208	-0.402
0.3	0.429	0.540	0.284	0.125	0.000
0.9	0.905	0.811	0.851	0.457	0.409

s.e.d. = 0.062

Table 5.7 Log_{10} mean Cd concentration (mg kg^{-1}) in shoot material for each Cd and $CaCO_3$ or S treatment for plants grown in the (a) Inman Valley and (b) Kapinnie soils.

(a) Inman Valley

	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
Cd treatment	0.96	0	0.84	2.24	3.64
0	-0.663	-0.287	-0.515	-0.432	-0.687
0.3	-0.235	-0.170	-0.037	-0.073	-0.356
0.9	0.029	0.211	0.271	0.282	0.104
2.7	0.437	0.673	0.818	0.828	0.651

s.e.d. = 0.118

(b) Kapinnie

	g S kg^{-1}		Control	g $CaCO_3$ kg^{-1}	
Cd treatment	0.34	0.17	0	0.35	0.70
0	-0.142	-0.101	-0.401	-0.332	-0.553
0.1	0.216	0.103	-0.096	-0.044	-0.066
0.3	0.414	0.239	0.091	0.087	-0.039
0.9	0.748	0.662	0.582	0.440	0.397

s.e.d. = 0.122

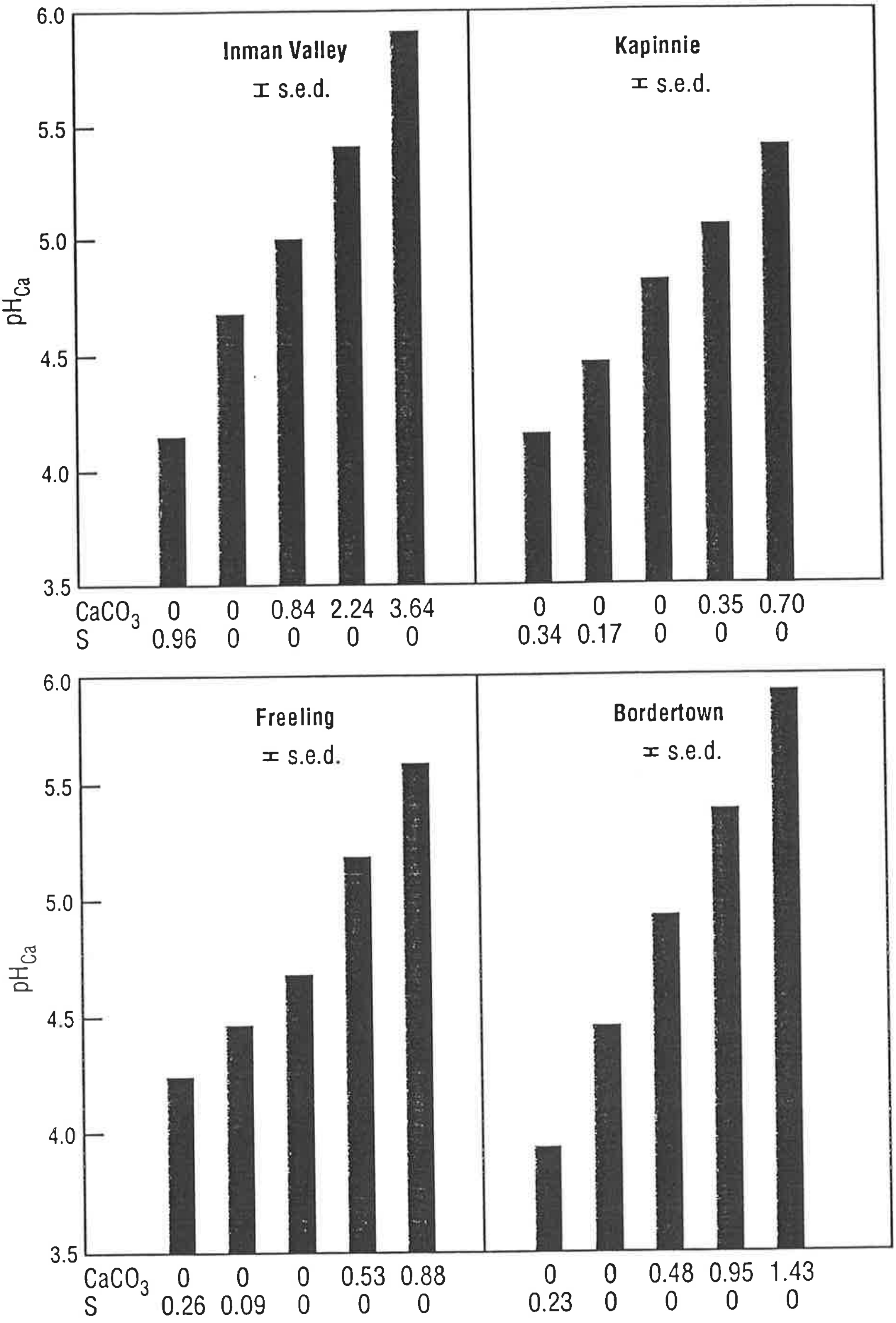


Figure 5.4. Mean pH values (1:5, 0.01M CaCl$_2$) obtained for each sulphur (S) or calcium carbonate (CaCO$_3$) treatment (g kg^{-1}).

D. Oliver, figure 5.4. D100/GER.

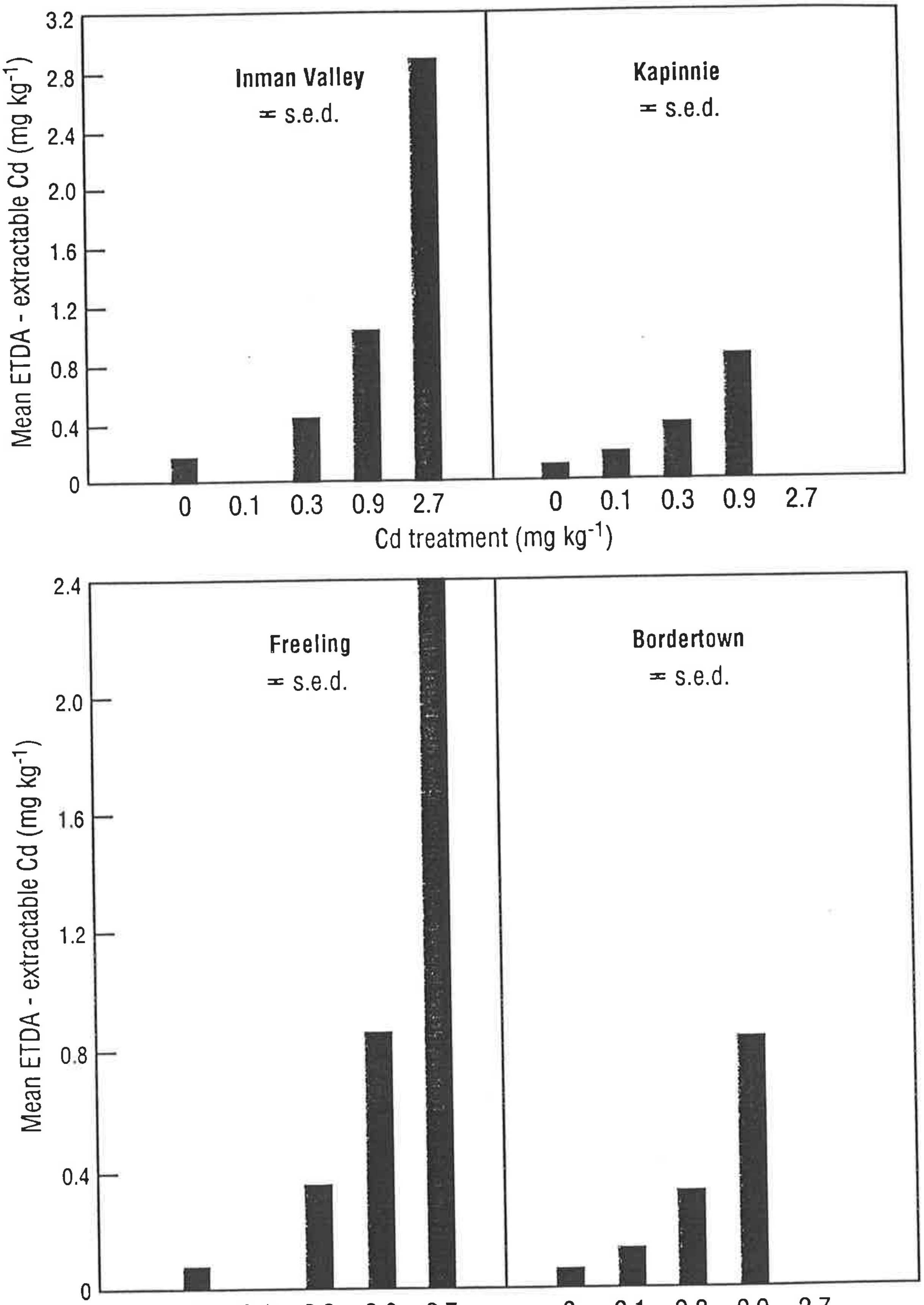


Figure 5.5. Mean EDTA - extractable Cd for all four soils for each Cd treatment.

D. Oliver, figure 5.5. D100/GER.

The $CaCl_2$-extractable Cd was affected by treatments in a manner similar to $Ca(NO_3)_2$-extractable Cd (Fig. 5.6).

5.4 DISCUSSION

5.4.1 Relationship Between Soil pH and Cd Concentration in Grain

Grain Cd concentrations were plotted against soil pH_{Ca} for each Cd treatment on each soil type (Figs. 5.7 - 5.10). Generally grain Cd concentrations decreased linearly with increasing soil pH except in grain for those plants grown in the Kapinnie and Inman Valley soils which had received the highest Cd treatment, Cd 0.9 and Cd 2.7, respectively (Figs. 5.7 and 5.10). In the case of grain from plants grown in the Kapinnie soil in the presence of high Cd (Cd 0.9), Cd concentration in grain decreased exponentially (Fig. 5.7). These results are in agreement with those found for pH effects on Cd concentration in wheat grain (Bingham *et al.*, 1979), maize (Street *et al.*, 1978) and fodder rape (Andersson and Nilsson, 1974). From adsorption studies (Forbes *et al.*, 1976; Tiller *et al.*, 1984) one would expect that raising soil pH would result in increased adsorption of Cd ions onto clay minerals and organic matter and therefore decreased availability of Cd for plant uptake.

The Cd concentration in grain from plants grown on the Inman Valley soil showed no change with increasing pH for treatments Cd 0, Cd 0.3 and Cd 0.9 (Fig. 5.10). For the highest Cd treatment (Cd 2.7) the grain Cd concentrations increased exponentially with increasing pH (Fig 5.10). Eriksson (1989) similarly found in a glasshouse study that while Cd concentrations in aerial parts of ryegrass plants generally decreased with increasing pH for all three Cd treatments (0, 1 and 5 mg kg^{-1}) for plants grown in a sandy soil, the Cd concentrations in plants grown in the clay soil varied between the pH treatments. Eriksson (1989) found increasing the initial pH of the clay soil from pH_w 5.0 to pH_w 6.0 had no effect on the Cd concentration in ryegrass plants in the first year and increased Cd concentration in ryegrass in the second year. Raising the pH_w to 7.0 however decreased the Cd concentration in ryegrass in both years.

The exponential increase in Cd concentration in grain for plants grown in the Inman Valley soil (Cd 2.7) is anomalous. The explanations provided by others for such anomalies, such as Ca ions displacing Cd ions from adsorption sites on clay particles (Andersson and Persson, 1982 in Eriksson, 1990) and increasing ionic strength decreasing adsorption to soil colloids (Petrucelli, 1985), do not apply to this particular case. The precipitation of CdS due to low redox in the Inman Valley soil is also unlikely because one would have expected a similar response to be seen for the other Cd treatments in the Inman Valley soil, which was not seen, and no H_2S was smelt from the Inman Valley soil which had received the highest Cd treatment (Cd 2.7). The soil pH of the Inman Valley soil may need to be raised to a higher value ($pH_{Ca} > 6.0$) before a decrease in grain Cd concentration is seen.

In this experiment all mean Cd concentrations in grain from plants grown in soil to which no Cd was added exceeded the MPC of 0.05 mg kg^{-1} and were much higher than Cd concentrations found in grain from field trials covering a range of soils (Chapter 6). This is most likely due to the restriction of root growth within the pots and the collection of soil for use in this experiment from the top 10 cm of the soil profile which would have a higher Cd concentration than soil taken from deeper in the profile.

The rates of change of and regression coefficients (r^2) for grain Cd concentrations and soil pH varied between soils. Grain concentrations from the sandy loam at Bordertown were the most responsive to increases in soil pH with r^2 values ranging from 0.65 to 0.88. The rate of change in concentration with increasing pH increased with higher Cd treatments for most soils (Table 5.10).

Table 5.8 Mean $Ca(NO_3)_2$ - extractable Cd (mg kg^{-1}) for each Cd and $CaCO_3$ or S treatment for (a) the Freeling soil and (b) the Bordertown soil.

(a) Freeling

Cd treatment	g S kg^{-1}		Control	g $CaCO_3$ kg^{-1}	
	0.26	0.09	0	0.53	0.88
0	0.028	0.022	0.019	0.009	0.007
0.3	0.123	0.081	0.104	0.078	0.021
0.9	0.330	0.240	0.180	0.118	0.079
2.7	0.950	0.700	0.500	0.315	0.076

s.e.d. = 0.021

(b) Bordertown

Cd treatment	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
	0.23	0	0.48	0.95	1.43
0	0.020	0.015	0.012	0.006	0.003
0.1	0.046	0.031	0.023	0.014	0.009
0.3	0.109	0.066	0.055	0.033	0.020
0.9	0.325	0.240	0.128	0.079	0.043

s.e.d. = 0.006

Table 5.9 Mean $Ca(NO_3)_2$ - extractable Cd (mg kg^{-1}) for each Cd and $CaCO_3$ or S treatment for (a) the Inman Valley soil and (b) the Kapinnie soil.

(a) Inman Valley

Cd treatment	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
	0.96	0	0.84	2.24	3.64
0	0.017	0.007	0.005	0.002	0.001
0.3	0.046	0.015	0.014	0.007	0.003
0.9	0.100	0.035	0.025	0.014	0.008
2.7	0.285	0.096	0.073	0.043	0.025

s.e.d. = 0.011

(b) Kapinnie

Cd treatment	g S kg^{-1}	Control	g $CaCO_3$ kg^{-1}		
	0.34	0.17	0	0.35	0.70
0	0.031	0.021	0.016	0.014	0.006
0.1	0.056	0.034	0.023	0.020	0.020
0.3	0.095	0.065	0.041	0.032	0.020
0.9	0.24	0.178	0.066	0.100	0.048

s.e.d. = 0.018

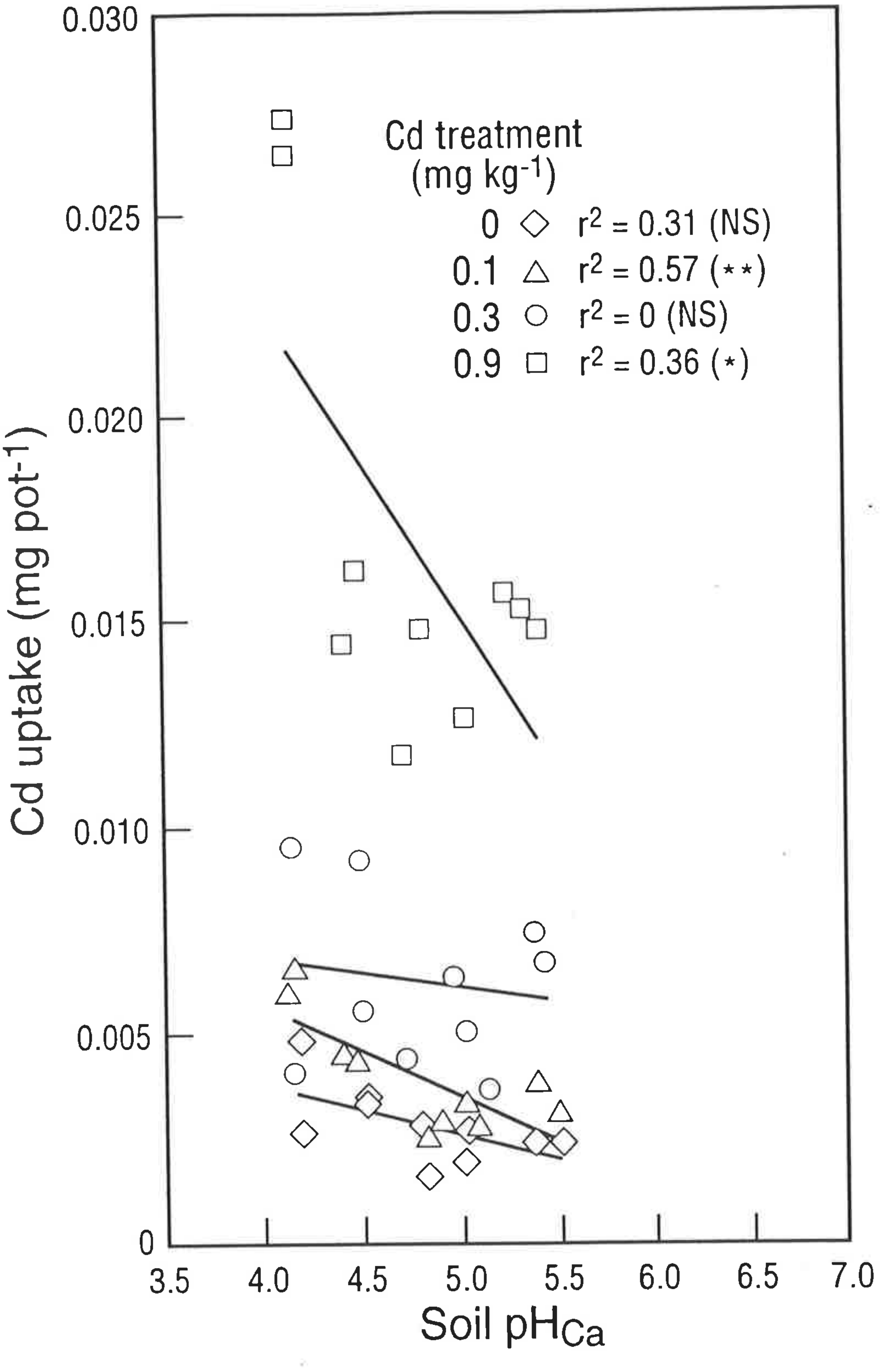


Figure 5.6. Relationship between Cd uptake (mg pot^{-1}) and soil pH for each Cd treatment applied to the Kapinnie soil.

D.Oliver, figure 5.6. D99/GER.

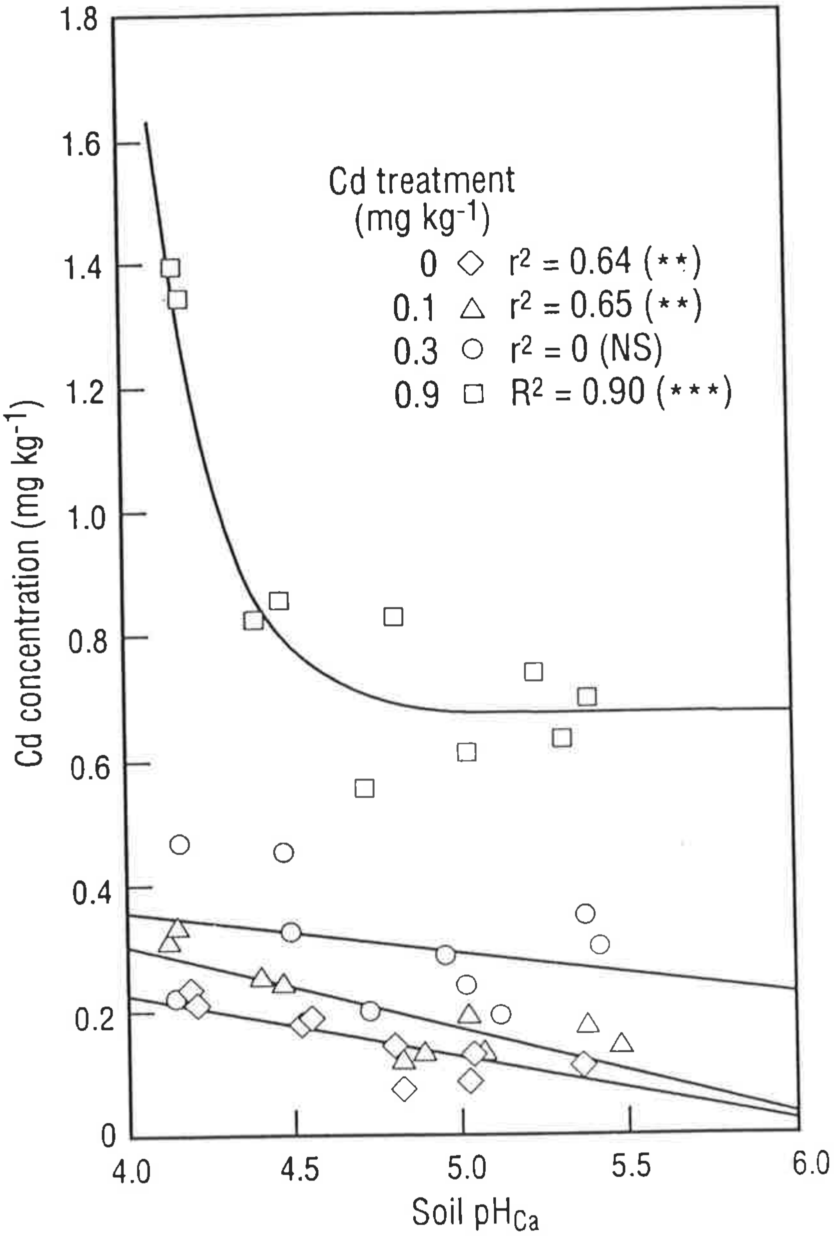


Figure 5.7. **Relationship between Cd concentration in grain (mg kg^{-1}) and soil pH for each Cd treatment applied to the Kapinnie soil.**

D.Oliver, figure 5.7. D99/GER.

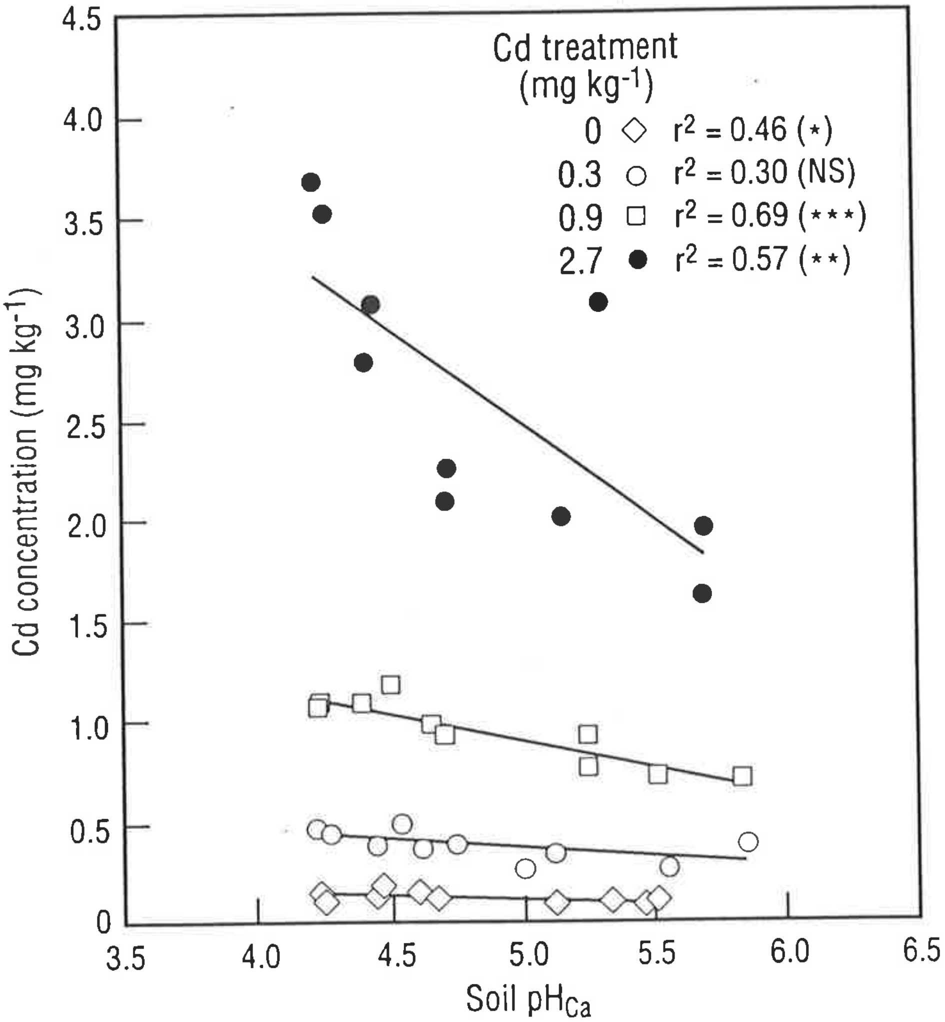


Figure 5.8. Relationship between Cd concentration in grain (mg kg^{-1}) and soil pH for each Cd treatment applied to the Freeling soil.

D.Oliver, figure 5.8. D99/GER.

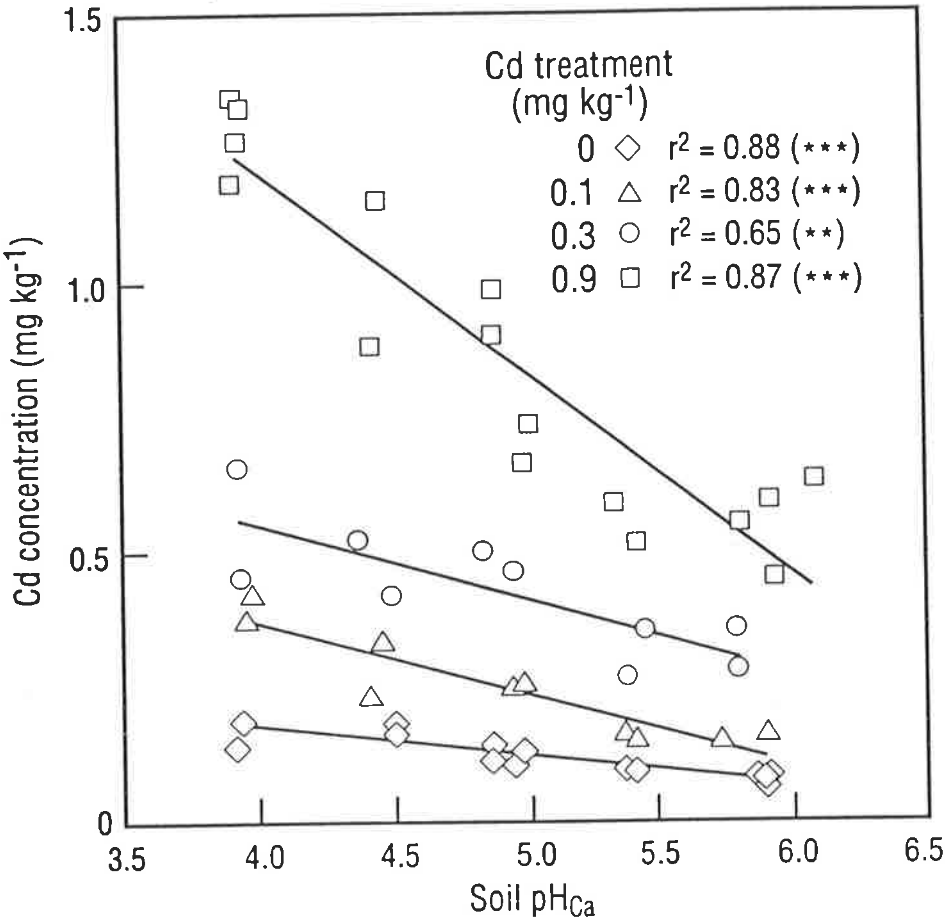


Figure 5.9. Relationship between Cd concentration in grain (mg kg^{-1}) and soil pH for each Cd treatment applied to the Bordertown soil.

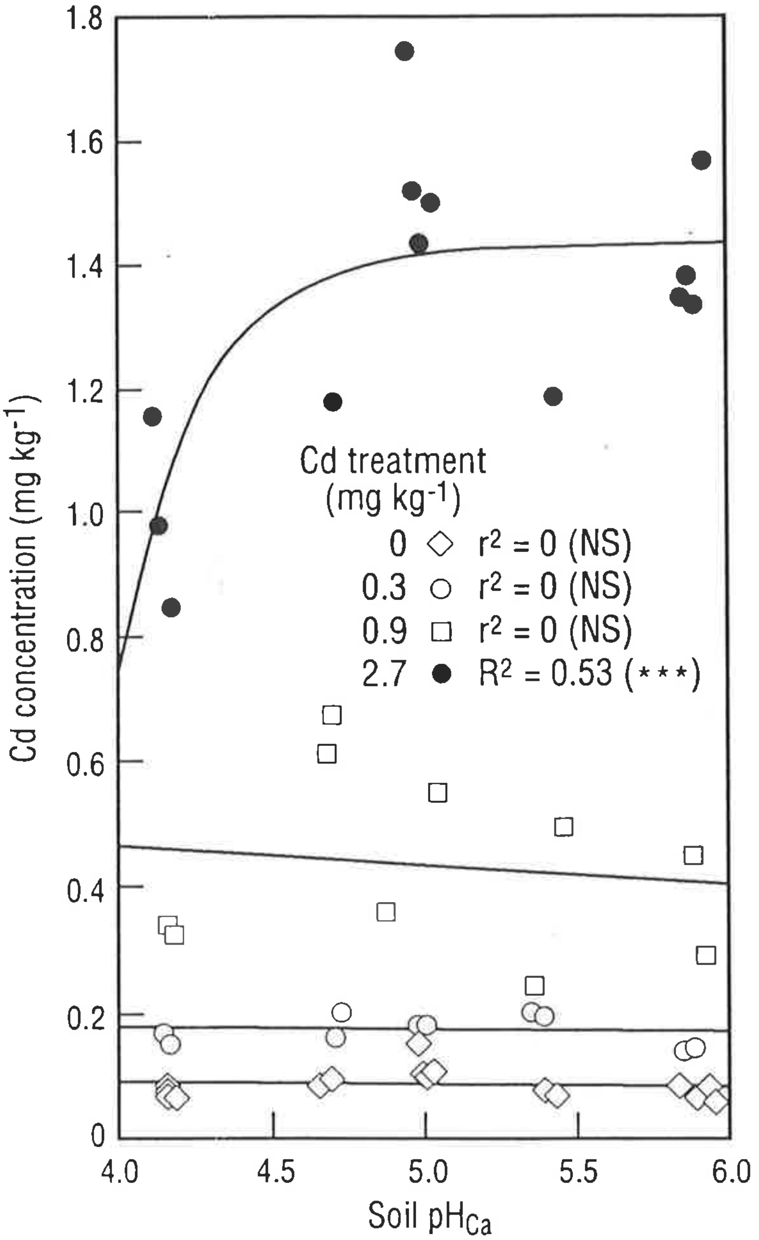


Figure 5.10. Relationship between Cd concentration in grain (mg kg^{-1}) and soil pH for each Cd treatment applied to the Inman Valley soil.

D.Oliver, figure 5.10. D99/GER.

While the grain Cd concentrations on the Kapinnie soil decreased significantly ($P<0.01$) with increasing pH for treatments Cd 0 and Cd 0.1 (r^2 = 0.64 and 0.65, respectively) the data for the Cd 0.3 treatment were widely scattered resulting in no significant relationship ($r^2 = 0$) (Fig. 5.7 and Table 5.10). An exponential curve was fitted to the data for the Cd 0.9 treatment and the coefficients are given in Appendix 5.6. Cadmium concentrations in grain increased significantly ($P<0.001$) at the highest S treatment but showed no statistical difference for the lime treatments (Fig. 5.7). The Cd concentration in grain from plants grown on the Freeling soil similarly showed no significant decreases with increasing pH ($r^2 = 0.30$) (Fig. 5.8 and Table 5.10) for Cd 0.3.

Overall the response of grain Cd concentrations to applied Cd and increases in soil pH varied in the order Bordertown > Kapinnie = Freeling >> Inman Valley (Table 5.10). This reflects variable affinity of the soils to bind Cd and hence decrease its availability for plant uptake which is related to clay content, organic matter content, kind of clay and surface area of clay. The absence of a decrease in grain Cd concentration with increasing pH on the Inman Valley soil was most likely due to a higher degree of Cd adsorption in this soil due to its high clay and organic matter content (Table 5.1). Although clay content provides no information on the presence of Fe- or Mn-oxides which provide specific adsorption sites for Cd (Tiller *et al.*, 1984), clay content does give an indication of the potential ability of a soil to bind Cd ions. Similarly, a high carbon content in the soil is an indication of potential adsorption sites for Cd within the soil.

Raising soil pH to minimise Cd uptake by wheat may in practice be effective only for medium to light textured soils. Although raising soil pH_{Ca} above 6.0 may have shown a decrease in grain Cd concentration on the Inman Valley soil this is unlikely because of the low slopes for the relationship between Cd concentration in grain and pH (Fig. 5.10 and Table 5.10). Also, this would not be an economically viable option for

Table 5.10 Change in Cd concentration (mg kg^{-1} per pH unit) in grain with increasing pH.

Cadmium treatment mg kg^{-1}	Soil											
	Inman Valley			Kapinnie			Freeling			Bordertown		
	Rate	n	r^2	Rate	n	r^2	Rate	n	r^2	Rate	n	r^2
0	-0.007	16	0 (NS)	-0.099	10	0.64 (**)	-0.046	10	0.46 (*)	-0.057	16	0.57 (***)
0.1	-	-	-	-0.134	10	0.65 (**)	-	-	-	-0.134	10	0.88 (***)
0.3	-0.008	10	0 (NS)	-0.069	10	0 (NS)	-0.097	10	0.30 (NS)	-0.144	10	0.83 (**)
0.9	-0.034	10	0 (NS)	-	-	-	-0.29	10	0.69 (***)	-0.38	16	0.64 (***)
2.7	-	-	-	-	-	-	-0.97	10	0.57 (**)	-	-	-

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, NS not significant

farmers because of the initial expense of applying sufficient quantities of lime to reach such a high pH and of the on-going expense of maintaining the pH_{Ca} above 6.0.

5.4.2 Relationship Between EDTA - extractable Soil Cd and Grain Cd Concentrations

On all soils and for all S and lime treatments grain Cd concentrations increased significantly ($P<0.001$) with increasing EDTA-extractable soil Cd concentrations (Table 5.11). However the rates of increase varied only slightly between the Kapinnie, Freeling and Bordertown soils. The rate of change on the Inman Valley soil was almost half that on the other three soils at comparable S or lime treatments (Table 5.11). This may possibly be explained by the greater adsorption of Cd onto the smectitic clay and organic matter in the Inman Valley soil (Table 5.1) compared with the other soils. This finding further explains the absence of a pH response in Cd concentration in grain from this soil. This suggestion could be investigated by adsorption studies.

5.4.3 Assessing the Impact of Growth Dilution Effects

A decrease in the concentration of any element due to a particular treatment may be due to the effect of the treatment on the availability of the element for plant uptake or due to the effect of the treatment on growth. When a treatment stimulates plant growth or increases yield the concentration of the element in the plant may be distributed throughout a greater biomass or a larger number of grains. The decrease in the concentration of the element in these latter cases is termed a dilution effect.

Distribution of Cd in grain

Cadmium uptake by wheat grain (mg pot^{-1}) was plotted against soil pH (Figs. 5.11 - 5.14) to assess whether the decrease in grain Cd concentration (mg kg^{-1}) was due to a dilution effect as the result of an increase in grain yield.

Only Cd uptake (mg pot^{-1}) data from the Cd 2.7 treatment on the Inman Valley soil changed with pH, increasing significantly ($P<0.01$) with increasing pH_{Ca} (Fig. 5.14). This trend was also seen with the grain Cd concentration (mg kg^{-1}) data from this Cd treatment on the Inman Valley soil. The Cd uptake data from Cd 0, Cd 0.3 and Cd 0.9 however did not change significantly with increasing pH.

Cadmium uptake data from both the Freeling (Cd 0 and Cd 0.3) and Kapinnie (Cd 0 and Cd 2.7) soils did not change significantly with increasing pH_{Ca}. However Cd uptake data from the Cd 0.1 and Cd 0.9 treatments on the Kapinnie soil and the Cd 0.3 and Cd 0.9 treatments on the Freeling soil decreased significantly ($P<0.01$, $P<0.05$, $P<0.05$ and $P<0.01$, respectively) with increasing pH_{Ca} (Figs. 5.11 and 5.12). Similarly, Cd uptake on the Bordertown soil decreased significantly ($P<0.001$, $P<0.01$ and $P<0.01$) for Cd 0, Cd 0.1 and Cd 0.9, respectively. Although the data for Cd 0.3 decreased with increasing pH_{Ca} the data was scattered and the regression was not significant (Fig. 5.13).

Thus Cd uptake (mg pot^{-1}) data from Kapinnie, Freeling and Bordertown showed the same trends with increasing pH as seen with grain Cd concentration (mg kg^{-1}) data. This indicates that the decreases seen in Cd concentrations were due to the effects of increasing pH on Cd concentrations in soil solution and hence Cd availability for plant uptake, and were not due to increasing pH decreasing yield. The decrease in Cd concentrations with increasing pH were not due to dilution effects on grain Cd concentrations.

Table 5.11 Change in Cd concentration in grain (mg kg^{-1}) with increasing EDTA-extractable soil Cd (mg kg^{-1}).

Inman Valley				Kapinnie			
Treatment (g kg^{-1} soil)	Rate	n	r^2	Treatment (g kg^{-1} soil)	Rate	n	r^2
0.96 S	+ 0.356	12	0.98 (***)	0.34 S	+ 1.59	8	0.93 (***)
Nil	+ 0.439	8	0.95 (***)	0.17 S	+ 0.91	8	0.97 (***)
0.84 $CaCO_3$	+ 0.533	12	0.98 (***)	Nil	+ 0.87	8	0.90 (***)
2.24 $CaCO_3$	+ 0.460	8	0.96 (***)	0.35 $CaCO_3$	+ 0.70	8	0.93 (***)
3.64 $CaCO_3$	+ 0.494	12	0.98 (***)	0.70 $CaCO_3$	+0.75	8	0.89 (***)

Freeling				Bordertown			
Treatment (g kg^{-1} soil)	Rate	n	r^2	Treatment (g kg^{-1} soil)	Rate	n	r^2
0.26 S	+ 1.46	8	0.99 (***)	0.23 S	+ 1.40	12	0.98 (***)
0.09 S	+ 1.27	8	1.00 (***)	Nil	+ 1.11	8	0.94 (***)
Nil	+ 0.86	8	0.99 (***)	0.48 $CaCO_3$	+ 0.86	12	0.91 (***)
0.53 $CaCO_3$	+ 1.08	8	0.91 (***)	0.95 $CaCO_3$	+ 0.57	8	0.95 (***)
0.88 $CaCO_3$	+ 0.71	8	0.97 (***)	1.43 $CaCO_3$	+0.59	12	0.95 (***)

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
NS not significant

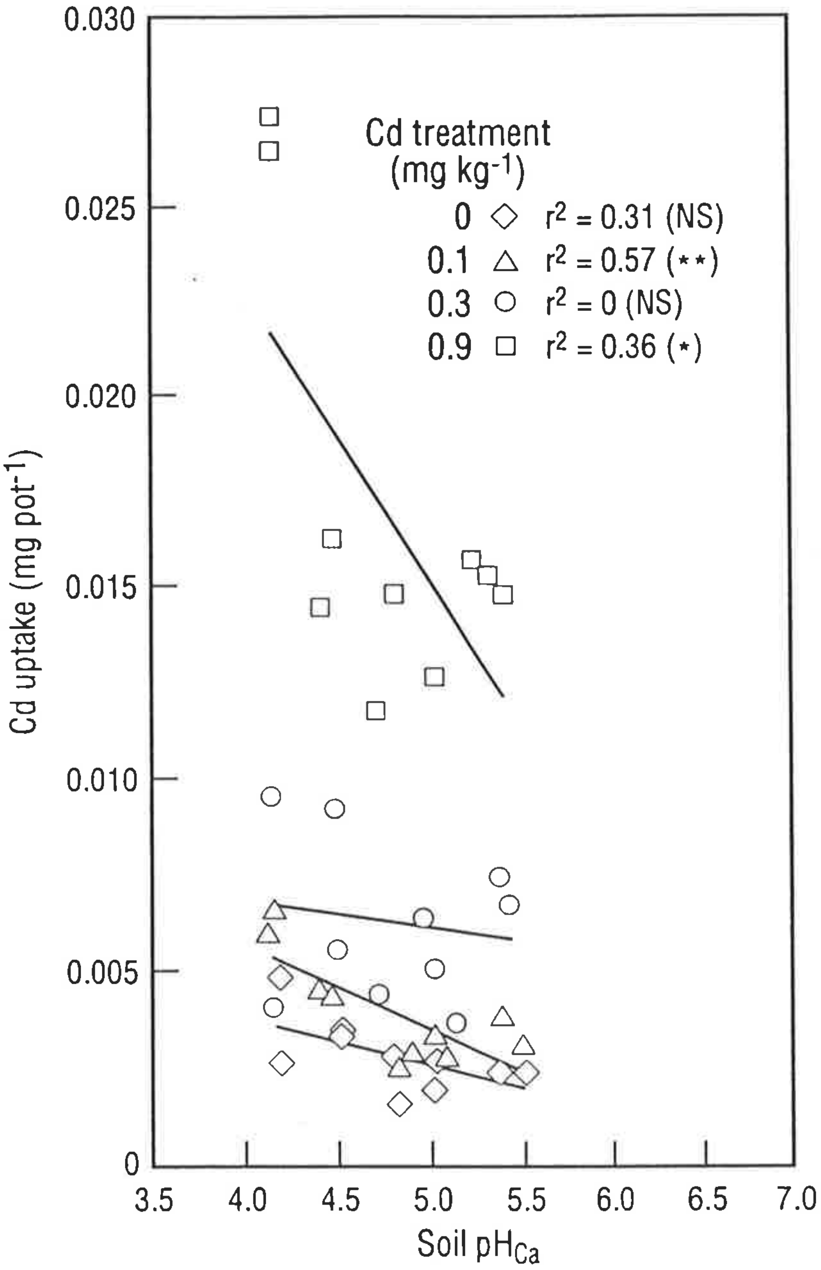


Figure 5.11. Relationship between Cd uptake by grain (mg pot^{-1}) and soil pH for each Cd treatment applied to the Kapinnie soil.

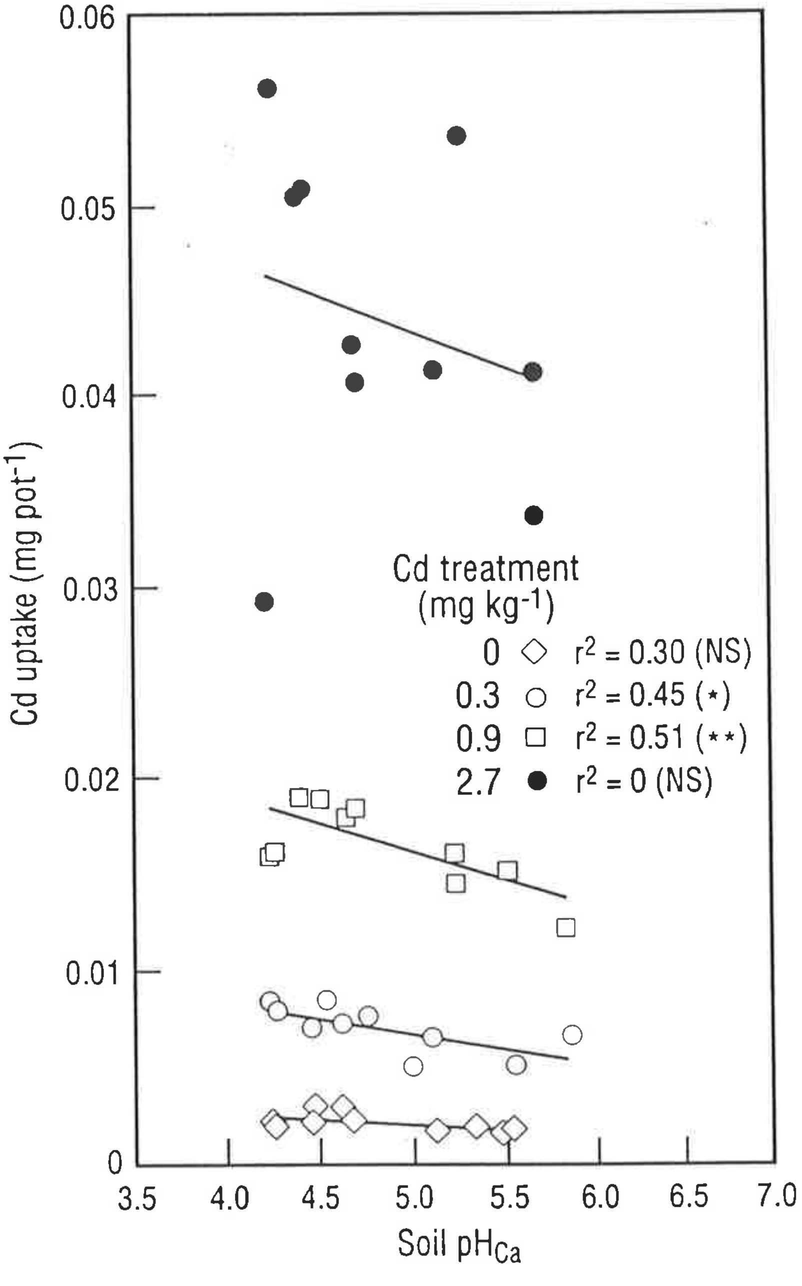


Figure 5.12. Relationship between Cd uptake by grain (mg pot^{-1}) and soil pH for each Cd treatment applied to the Freeling soil.

D.Oliver, figure 5.12. D99/GER.

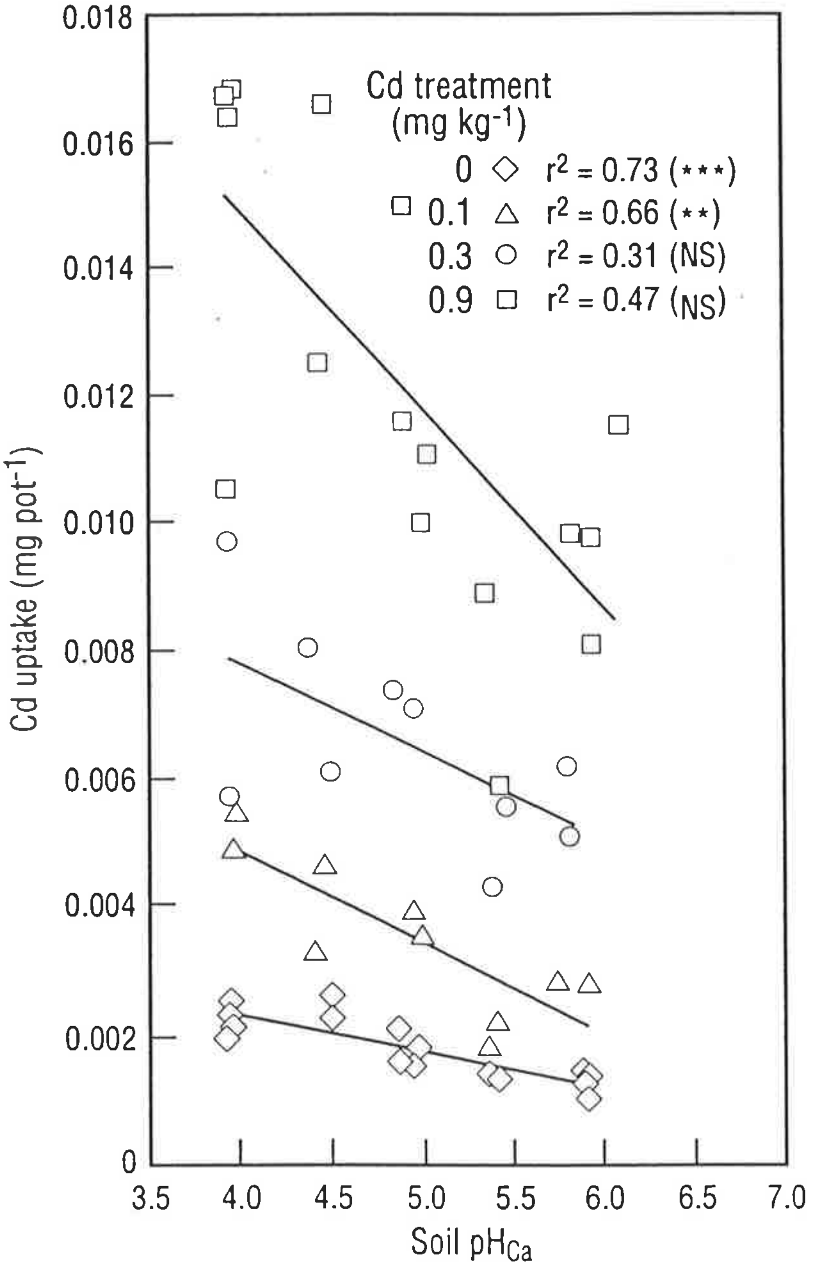


Figure 5.13. Relationship between Cd uptake by grain (mg pot^{-1}) and soil pH for each Cd treatment applied to the Bordertown soil.

D.Oliver, figure 5.13. D99/GER.

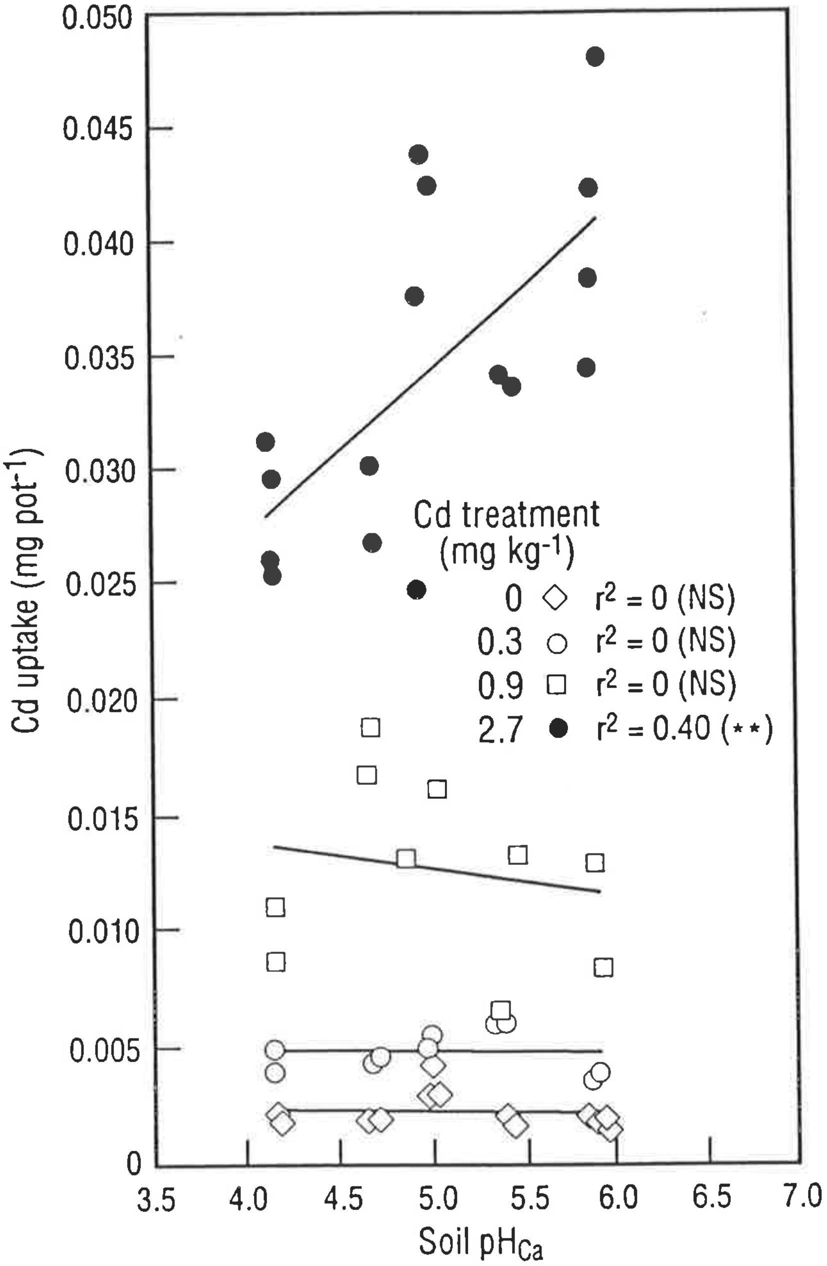


Figure 5.14. Relationship between Cd uptake by grain (mg pot^{-1}) and soil pH for each Cd treatment applied to the Inman Valley soil.

D.Oliver, figure 5.14. D99/GER.

Distribution of Cd in shoots

Changes in Cd concentration in grain with S and lime treatments may be due to changes in the distribution of Cd in the plant material for each treatment. Consequently, the relationship between the ratio of Cd concentration in shoot material (excluding grain) : Cd concentration in grain (Cd shoot : Cd grain) with soil pH was determined.

Neither Cd treatment nor treatments to adjust pH had significant effects on the ratio of Cd shoot : Cd grain for plants grown on the Kapinnie soil. Treatments to adjust pH had significant ($P<0.05$ and $P<0.001$, respectively) effects on the ratio of Cd shoot : Cd grain for plants grown on the Inman Valley and Bordertown soils (Table 5.12a and 5.12c). The Cd shoot : Cd grain ratio for the Inman Valley soil increased with increasing $CaCO_3$ up to the 2.24 g $CaCO_3$ kg^{-1} soil treatment; further increase to 3.64 g kg^{-1} decreased the ratio back to a value that did not (Table 5.12a). On the Bordertown soil the Cd shoot : Cd grain ratio varied with the S and $CaCO_3$ treatments in an irregular manner and showed no trend (Table 5.12c). The Cd shoot : Cd grain ratio increased significantly ($P<0.001$) on the Freeling soil with increasing Cd treatment (Table 5.12b). On the Bordertown soil the ratio generally increased significantly ($P<0.001$) at only the highest Cd treatments for the S, control and $CaCO_3$ treatments (Table 5.12c).

If the lime and/or S treatments were altering the distribution of Cd within the plant such that the decrease in Cd concentration in grain was the result of an increase in the proportion of Cd in the shoot material one would expect to observe an increase in the ratio of Cd shoot : Cd grain. However, in the case of plants grown on the Kapinnie and Freeling soils there was no significant change in the ratio with lime or S treatments. Thus, the significant ($P<0.001$) decrease in grain Cd concentration with increasing lime and decreasing S treatments for plants grown on the Kapinnie and Freeling soils was not due to a growth dilution effect.

In the case of the plants grown on the Bordertown soil the ratio showed no change with increasing pH for the Cd 0 treatment. For the other Cd treatments the ratio varied in a complicated manner (Table 5.12c) suggesting the decrease in Cd concentration in grain in plants grown on the Bordertown soil is unlikely to be due to a growth dilution effect.

Since the ratio varies in a complicated manner with the different treatments this would suggest that you cannot reasonably expect to get a clear relationship between Cd concentration in grain and extractable soil Cd. This implies that you would be unable to predict Cd in grain from the extractable soil Cd.

5.5 CONCLUSIONS

Cadmium concentrations in grain increased significantly ($P<0.001$) with increasing Cd treatments for all soils. Generally raising soil pH_{Ca} from 4.0 to 6.0 decreased the Cd concentration in grain significantly ($P<0.001$) at all Cd treatments on the Bordertown soil (Fig. 5.9). On the Freeling soil a significant ($P<0.001$) decrease in Cd concentration in grain was seen only in the highest Cd treatment (Cd 2.7) (Fig. 5.8). While the S treatments, which decreased the pH_{Ca} from 4.8 to 4.2, significantly ($P<0.001$) increased the Cd concentration in grain grown on the Kapinnie soil, the lime treatments, which increased pH_{Ca} from 4.8 to 5.4, had no significant effects at all Cd treatments (Fig. 5.7). Generally the Cd concentrations in grain grown on the Inman Valley soil showed negligible responses to changes in pH_{Ca} at all Cd treatments. The main exception was the anomalous increase in Cd concentration in grain when the pH_{Ca} was raised from 4.15 to 4.7 at the highest Cd treatment (Cd 2.7) (Fig. 5.10). For all four soils the lime and sulphur treatments had no significant effect on Cd concentration in grain when no Cd was added to the soil. The EDTA extraction removed nearly all of the added Cd from each soil despite equilibrating the soils for several months.

This study suggests that while raising soil pH_{Ca} from 4.0-4.2 to 4.5-4.8 would decrease the Cd concentration in grain grown in medium to light textured soils, it is not a very effective method for minimising Cd concentration in grain grown in heavier textured soils. Further the cost of applying lime in certain areas would prohibit farmers from using lime to raise soil pH_{Ca} above 5.0.

Table 5.12 Mean ratio Cd concentration in shoot material (excluding grain): Cd concentration in grain (Cd shoot: Cd grain) for plants grown on the (a) Inman Valley, (b) Freeling and (c) Bordertown soils.

(a) Inman Valley

Treatment (g kg^{-1})	S	Control	C_aC0_3		
	0.96	0	0.84	2.24	3.64
Mean Cd shoot: Cd grain[1]	3.16 : 1 (1.16 : 0.37)	4.12 : 1 (1.91 : 0.55)	4.12 : 1 (2.42 : 0.56)	5.18 : 1 (2.48 : 0.49)	3.28 : 1 (1.64 : 0.48)

s.e.d. = 0.6

(b) Freeling

Cd Treatment (mg kg^{-1})	0	0.3	0.9	2.7
Mean Cd shoot : Cd grain	3.66 : 1 (0.43 : 0.12)	4.57 : 1 (1.68 : 0.37)	5.09 : 1 (4.75 : 0.93)	6.51 : 1 (16.13 : 2.59)

s.e.d. = 0.3

(c) Bordertown

Cd treatment (mg kg^{-1})	g S kg^{-1} 0.23	Control 0	g $CaC0_3$ kg^{-1}		
			0.48	0.95	1.43
0	3.6 : 1 (0.66 : 0.17)	3.7 : 1 (0.60 : 0.16)	3.3 : 1 (0.37 : 0.12)	3.6 : 1 (0.30 : 0.09)	3.1 : 1 (0.19 : 0.07)
0.1	4.0 : 1 (1.58 : 0.44)	4.5 : 1 (1.19 : 0.29)	2.9 : 1 (0.72 : 0.26)	4.4 : 1 (0.63 : 0.17)	2.8 : 1 (0.40 : 0.15)
0.3	4.9 : 1 (2.74 : 0.54)	7.5 : 1 (3.51 : 0.45)	4.2 : 1 (1.92 : 0.45)	4.6 : 1 (1.34 :0.28)	3.3 : 1 (1.00 : 0.29)
0.9	6.2 : 1 (8.03: 1.28)	6.6 : 1 (6.47 : 0.98)	10.3 : 1 (7.14 : 0.81)	5.3 : 1 (2.88 : 0.59)	5.3 : 1 (2.57 : 0.54)

s.e.d. = 0.8

1. Actual concentrations (mg kg^{-1}) are given in parentheses.

CHAPTER 6

EFFECTIVENESS OF LIMING TO MINIMISE CADMIUM CONCENTRATION IN WHEAT GRAIN GROWN IN THE FIELD.

6.1 INTRODUCTION

In Chapter 5, the effectiveness of lime in minimising Cd uptake by wheat grown in the glasshouse was studied. However, concentrations of metals are often much higher in glasshouse-grown than in field-grown plants. This is usually attributed to different environmental conditions, to the small volume of soil available to the roots and the fact that the treatments are mixed through all the soil. Consequently the plant is "forced" to take up the metal, while in the field the plant roots may be able to grow beyond the region of high metal concentration. As discussed in Chapter 5 some researchers (Andersson and Nilsson, 1974; Williams and David, 1976; Street *et al.*, 1978; Bingham *et al.*, 1979) have found that raising soil pH decreased Cd concentration in plants grown in the glasshouse while similar responses have not been found in the field (Pepper *et al.*, 1983). An extensive survey of 124 oat grain and soil samples across Sweden found soil pH accounted for only 24% of the variation in grain Cd concentration (Eriksson, 1990). Jaakkola (1977) failed to find any clear effects of lime application on grain Cd concentration in spring wheat in both glasshouse pot experiments and in field experiments.

There is little information in the literature on the effects of lime on Cd uptake in the field. Those field studies that have been reported have usually been conducted on soils with elevated Cd concentrations, generally due to applications of sewage sludge. Consequently, a study was made on the influence of soil pH on Cd uptake by wheat on several soil types in the field and where the Cd concentrations were not elevated.

6.2 MATERIALS AND METHODS

6.2.1 Experimental Outline

Grain and soil samples from eight liming/sulphur experiments that were established in 1987 formed the basis of this study. The experiments were established to investigate the amelioration of acid agricultural soils in south-east Australia with a view to improving the prediction of yield response to lime by crop and pasture plants on acid soils.

Four experiments were established by the Victorian Department of Agriculture (DAV) in the Rutherglen district of Victoria, at Devenish (Experiment 6.1), Rutherglen (Experiment 6.2), Burramine (Experiment 6.3) and Lake Rowan (Experiment 6.4) and four were established by the New South Wales Department of Agriculture and Fisheries (NSW DAF) in the Wagga Wagga district of New South Wales, at Borambola (Experiment 6.5), Brucedale (Experiment 6.6), Mangoplah (Experiment 6.7) and Book Book (Experiment 6.8). Details of the chemical and physical characteristics of the soils (0 - 10 cm) at each site are given in Table 6.1.

Wheat cv. Matong, barley cv. Schooner and triticale, (*Triticosecale* spp.) cv. Currency were sown at all sites in 1987, 1988 and 1989, but only wheat was analysed in this study.

The treatments, which ranged from 0 - 1.5 t sulphur ha^{-1} and 0 - 6 t lime ha^{-1} (Tables 6.2 and 6.3), were randomised in four replicate blocks, and mixed thoroughly through the soil (0-10 cm) using a rotary hoe. The sulphur and lime treatments were applied prior to sowing in 1987. The plots were 15 m x 2 m (Experiments 6.1 - 6.4), 15 m x 1.3 m (Experiments 6.6 - 6.8) and 11 m x 1.3 m (Experiment 6.5) in size.

6.2.2 Soil Measurements

Wheat grain from two replicates of each experiment was analysed for Cd as detailed in Chapter 3.

Cores of soil to a depth of 10 cm were collected during September and October each year across the plots in each experiment. They were bulked, sieved to pass a 2 mm mesh screen and mixed well before being sub-sampled. EDTA soil extractions were

Table 6-1 Selected chemical and physical characteristics and classification of soil (0-10 cm) from Experiments 6.1-6.4 (Rutherglen, Vic.) and Experiments 6.5-6.8 (Wagga Wagga, N.S.W.)

Experiment	Location	Great Soil Group [A]	Soil Texture [B]	% Clay <0.002 mm	% Silt 0.002-0.02 mm	% Sand 0.02-2.0 mm	pH (1:5, 0.01M $CaCl_2$) (April/May 1987)	Total C [C] %	HCO_3 [D] -P ($mgkg^{-1}$)	Range EDTA-extractable Cd ($mgkg^{-1}$) (Aug/Sept 1987)
6.1	Devenish 145° 51'W 36° 21'S	red podzolic solodic	sandy loam	13	43	45	4.25	1.32	41	0.037-0.086
6.2	Rutherglen 146° 28'W 36° 08'S	podzolised red earth	fine sandy loam	10	22	68	4.25	1.71	42	0.049-0.123
6.3	Burramine 145° 53'W 36° 04'S	red brown earth	fine sandy loam	16	30	58	4.70	1.36	44	0.049-0.098
6.4	Lake Rowan 145° 48'W 36° 12'S	solodic	sandy loam	15	19	69	4.73	1.49	21	0.012-0.049
6.5	Borambola 147° 31'W 35° 13'S	yellow solodic	sandy loam	8	19	75	4.15	1.10	34	0.036-0.123
6.6	Brucedale 147° 25'W 35° 02'S	calcareous red earth	sandy loam	19	14	69	4.33	1.17	15	0.036-0.073
6.7	Mangoplah 147° 15'W 35° 23'S	yellow solodic	sandy loam	9	25	68	4.44	1.28	28	0.036-0.073
6.8	Book Book 147° 35'W 35° 24'S	podzolised red earth	sandy loam	12	21	70	4.66	1.08	34	0.036-0.061

A. Stace et. al., (1968)
B. Northcote, (1974)
C. Merry and Spouncer, (1988)
D. Colwell, (1965)

made on the two replicates corresponding to the grain analysis. Details of the methodology are given in Chapter 3. Soil pH measurements were made on all replicates by the Chemistry Laboratories in the DAV and the NSW DAF for Experiments 6.1 - 6.4 and 6.5 - 6.8 (1987), respectively. pH measurements were repeated to confirm the method used in our laboratory gave the same results as obtained by the DAV.

Total organic carbon was determined by combustion. A weighed sub-sample of soil was ignited in a stream of oxygen at 1050-1200°C. The water vapour was removed and the amount of carbon dioxide released was measured by an infra-red detector (Merry and Spouncer, 1988). The Colwell (1965) method was used to determine the bicarbonate extractable phosphorus content of the soils. Both the carbon and phosphorus measurements were made by the respective Chemistry Laboratories in the DAV and the NSW DAF for Experiments 6.1 - 6.4 and 6.5 - 6.8, respectively.

6.3 RESULTS

6.3.1 Cd Concentration in Grain

The effects of the sulphur and lime treatments on Cd concentration in grain were determined by analysis of variance. The data for each experiment were then fitted to an exponential model with the asymptote, a, set to zero, and statistical differences between curves were calculated as outlined in Chapter 3. In some cases the data did not fit an exponential model very well. A significant reduction in the error mean square could be obtained by allowing the asymptote, a, to be estimated. However, in these cases the model was numerically unstable. Parameters for the exponential model for each experiment are given in Table 6.4 and the percent variance accounted for by the model is given in brackets for each year in Figs. 6.1 - 6.4. The soil pH at which half the observed decrease in Cd concentration ($ODCd_{50}$) occurred was calculated for those experiments where treatments had significant effects on the grain Cd concentration. $ODCd_{50}$ was calculated by [pH_{Ca} 4.0 + (ln 2/ln k)] (Table 6.4).

Generally, the Cd concentration in grain decreased with increasing rates of lime and decreasing rates of sulphur. Significant decreases in concentration with increasing rates of lime were seen in Experiments 6.2 ($P<0.001$ 1988 only), 6.3 ($P<0.01$ in 1987 and $P<0.05$ in 1988), 6.5 ($P<0.01$ in 1987 and $P<0.05$ in 1989), 6.6 ($P<0.05$ in 1987 and 1988; $P<0.001$ in 1989), 6.7 ($P<0.05$ in 1987 and $P<0.01$ in 1988) and 6.8 ($P<0.001$ in 1987 and $P<0.05$ in 1988). The mean Cd concentrations in grain for each treatment for Experiments 6.1 to 6.4 and 6.5 to 6.8 are given in Tables 6.5 and 6.6, respectively.

6.3.2 Soil Factors

pH

The lime and sulphur treatments had highly significant ($P<0.001$) effects on soil pH in all experiments except Experiment 6.4, and produced a range of pH_{Ca} values from approximately 4 to 6. In Experiment 6.4 the effects were only just significant ($P<0.05$) in 1987 and not significant in 1988. The soil pH values generally increased with increasing rates of lime and decreased with increasing additions of sulphur in most experiments (Tables 6.2 and 6.3).

EDTA - extractable soil Cd

Neither lime nor sulphur treatments had significant effects on EDTA - extractable Cd concentrations (mg kg^{-1}) in the soil in any experiment. The ranges in EDTA - extractable Cd concentrations for all treatments and replicates were 0.037 - 0.086 mg kg^{-1} (Experiment 6.1); 0.049 - 0.123 mg kg^{-1} (Experiment 6.2); 0.049 - 0.098 mg kg^{-1} (Experiment 6.3); and 0.012 - 0.049 mg kg^{-1} (Experiment 6.4) (Table 6.1). The range of EDTA-extractable Cd concentrations in Experiments 6.5 - 6.8 was also large and the values in all 4 experiments ranged from 0.036 to 0.123 mg kg^{-1} (Table 6.1).

Table 6-2 Soil pH values (1:5, 0.01M $CaCl_2$) for the lime and sulphur treatments in Experiments 6.1 to 6.4 in the Rutherglen district

Experiment		Year	Treatment							
No.	Site		t S ha^{-1}		Control	t lime ha^{-1}				s.e.d.[1]
			0.75		**0**	**0.75**	**1.50**	**3.00**	**6.00**	
6.1	Devenish	1987	4.42	-	4.51	4.63	4.98	5.24	5.70	0.05
		1988	4.06	-	4.14	4.38	4.91	5.52	6.48	0.08
			0.60							
6.2	Rutherglen	1987	4.43	-	4.54	4.67	5.12	5.31	6.32	0.09
		1988	4.03	-	4.16	4.38	4.84	5.32	6.20	0.07
			1.67	**0.83**						
6.3	Burramine	1987	4.74	4.72	4.94	4.99	5.34	5.66	-	0.05
		1988	4.30	4.40	4.68	4.86	5.37	5.89	-	0.08
			1.5	**0.75**						
6.4	Lake Rowan	1987	4.72	4.52	5.00	4.91	5.37	5.76	-	0.18
		1988	5.21	4.58	5.62	5.07	5.12	5.20	-	0.45

[1] s.e.d. in this and subsequent tables, unless stated otherwise, is the standard error of difference of means = $\sqrt{\text{error}}$ MS $\sqrt{\frac{2}{n}}$

Table 6-3 Soil pH values (1:5, 0.01M $CaCl_2$) for the lime and sulphur treatments in Experiments 6.5 to 6.8 in the Wagga Wagga district

Experiment		Year	Treatment							
No.	Site		t S ha^{-1}		Control	t lime ha^{-1}				s.e.d.
			0.48	0.24	0	0.75	1.50	3.00	6.00	
6.5	Borambola	1987	-	4.09	4.16	4.73	5.25	5.85	6.26	0.13
		1988	-	4.01	4.07	4.54	4.83	5.69	6.54	0.08
		1989	-	4.10	4.19	4.30	4.52	5.36	6.16	0.11
6.6	Brucedale	1987	4.02	4.07	4.29	4.67	4.90	5.43	-	0.07
		1988	3.92	3.96	4.22	4.46	4.69	5.22	-	0.10
		1989	4.02	3.87	4.09	4.30	4.43	5.28	-	0.07
6.7	Mangoplah	1987	4.07	4.34	4.40	4.81	5.24	5.84	-	0.06
		1988	4.16	4.30	4.34	4.65	5.00	5.43	-	0.06
		1989	3.98	3.99	4.21	4.38	4.55	5.29	-	0.06
6.8	Book Book	1987	4.12	4.17	4.27	4.57	4.57	5.49	-	0.20
		1988	4.04	4.07	4.29	4.64	5.00	5.75	-	0.08
		1989	4.08	3.93	4.19	4.30	4.59	5.35	-	0.05

Table 6.4 Exponential equations for the relationship between grain Cd concentration (mg kg^{-1}) and pH for each experiment. The equation is of the form:
grain Cd concentration $= b * \exp(^{-k})^{pH}$

Experiment	Year	b	Exp^{-k}	$ODCd_{50}$[1]	Percent Variance Accounted For By Model[2]
6.1	1987	0.150	0.732	-	27.1
	1988	0.027	0.978	-	-
6.2	1987	0.126	0.716	-	32.3
	1988	0.106	0.705	6.00	29.5
6.3	1987	1.060	0.495	5.00	55.5
	1988	0.424	0.501	5.00	64.2
6.5	1987	0.036	0.984	-	-
	1988	0.068	0.821	-	30.2
	1989	0.293	0.698	5.90	64.2
6.6	1987	0.135	0.659	4.66	51.6
	1988	0.071	0.740	6.30	15.7
	1989	0.073	0.832	7.77	8.0
6.7	1987	23.500	0.212	4.45	57.6
	1988	0.179	0.595	5.34	39.5
	1989	0.141	0.685	-	36.3
6.8	1987	0.225	0.599	5.35	25.4
	1988	0.040	0.817	7.43	8.8
	1989	0.042	0.893	-	-

[1] $ODCd_{50}$ = pH at which half the observed decrease in Cd concentration in grain occurred
= $pH_{Ca}4.0 + (\ln 2/\ln k)$

$ODCd_{50}$ was calculated only for those experiments where the lime and sulphur treatments had significant effects on Cd concentrations in grain.

[2] When the model did not fit the data no value is given for the percent variance accounted for by the model.

Table 6-5 Mean Cd concentrations in grain (mg kg^{-1}) for the lime and sulphur treatments in Experiments 6.1 to 6.4 in the Rutherglen district

Experiment		Year	Treatment							
No.	Site		t S ha^{-1}		control	t lime ha^{-1}				s.e.d.
			0.75		0	0.75	1.50	3.00	6.00	
6.1	Devenish	1987	0.043	-	0.029	0.034	0.031	0.027	0.027	0.005
		1988	0.030	-	0.021	0.022	0.022	0.031	0.020	0.004
			0.60							
6.2	Rutherglen	1987	0.035	-	0.029	0.020	0.021	0.017	0.020	0.008
		1988	0.031	-	0.027	0.019	0.014	0.014	0.018	0.004
			1.67	0.83						
6.3	Burramine	1987	0.043	0.040	0.026	0.028	0.024	0.024	-	0.003
		1988	0.023	0.022	0.013	0.014	0.011	0.008	-	0.002
			1.5	0.75						
6.4	Lake Rowan	1987	0.030	0.025	0.028	0.032	0.026	0.017	-	0.004
		1988	0.037	0.044	0.024	0.034	0.031	0.023	-	0.005

Table 6-6 Mean Cd concentrations in grain (mg kg^{-1}) for the lime and sulphur treatments in Experiments 6.5 to 6.8 in the Wagga Wagga district

Experiment		Year	Treatment							
No.	Site		t S ha^{-1}		control	t lime ha^{-1}				s.e.d.
			0.48	0.24	0	0.75	1.50	3.00	6.00	
6.5	Borambola	1987	-	0.029	0.037	0.034	0.031	0.030	0.035	0.004
		1988	-	0.028	0.032	0.032	0.029	0.019	0.018	0.005
		1989	-	0.077	0.069	0.052	0.053	0.040	0.037	0.006
6.6	Brucedale	1987	0.030	0.024	0.019	0.018	0.016	0.017	-	0.005
		1988	0.019	0.022	0.022	0.020	0.021	0.010	-	0.004
		1989	0.043	0.034	0.033	0.031	0.030	0.028	-	0.004
6.7	Mangoplah	1987	0.044	0.027	0.020	0.014	0.012	0.012	-	0.007
		1988	0.025	0.016	0.019	0.014	0.012	0.013	-	0.002
		1989	0.032	0.030	0.032	0.025	0.023	0.021	-	0.003
6.8	Book Book	1987	0.030	0.036	0.018	0.021	0.017	0.015	-	0.003
		1988	0.018	0.019	0.016	0.014	0.013	0.015	-	0.003
		1989	0.026	0.023	0.026	0.029	0.029	0.021	-	0.004

6.4 DISCUSSION

6.4.1 Cd Concentration in Grain

Since the lime and sulphur treatments significantly ($P<0.001$) affected soil pH, grain Cd concentrations were plotted against pH. Examples are shown for Experiments 6.2, 6.5, 6.6 and 6.7 in Figs. 6.1 - 6.4. (Data for Experiments 6.1, 6.3 and 6.8 are in Appendices 6.1-6.3. Since there were no significant effects of the lime or sulphur treatments on Cd concentrations in grain from Experiment 6.4 no data are shown for this experiment). The Cd concentration in grain violated the MPC of 0.05 mg kg^{-1} only in Experiment 6.5 (1989) (Fig. 6.2). However raising the pH_{Ca} above 5.0 decreased the Cd concentration in grain below the MPC.

These studies demonstrate large variations in Cd concentration in wheat grain between sites and between years. Treatments which raised the soil pH_{Ca} from 4.0 to 6.0 generally decreased the Cd concentration in wheat grain (Figs. 6.1 - 6.4). Generally, concentrations decreased exponentially with little further decrease occurring as the pH_{Ca} increased above approximately 5.0. Mortvedt *et al.* (1981) similarly found lower Cd concentrations in wheat grain grown on a silty loam that had been limed to achieve a pH of approximately 6.0 compared with adding lime to the soil to reach a pH of approximately 5.0. No details were given by Mortvedt *et al.* (1981) about the method used to measure pH. As in this study, their anthropogenic source of Cd was phosphate fertilizer. However Mortvedt *et al.* (1981) used three sources of diammonium phosphate fertilizer with varying concentrations of Cd. Pepper *et al.* (1973) studied the effects of liming two soils, a silty loam and a fine sandy loam which had both received sewage sludge, on Cd uptake by maize in two subsequent years. In contrast to Mortvedt *et al.* (1981), they found liming did not appreciably decrease Cd concentrations in the plants. However, the results of Pepper *et al.* (1973) were confounded by the addition of metals combined in the sludge, which probably controlled the Cd availability in the early years of their study, and may have masked the effects of soil factors on availability. Corey *et al.* (1987) concluded that specific adsorption of metals by the sludge would normally be the factor controlling availability of metals in some sludge-soil mixtures.

In some experiments, no change in Cd concentration in grain was found with increases in soil pH, e.g. the 1987 harvest of grain from Experiment 6.5 (Fig. 6.2). Since the same wheat variety was grown at every site every year the lack of response in some years cannot be attributed to varietal differences.

Variation in Cd concentration in grain between years

The variation in Cd concentration in grain between years was negligible in a few experiments, while in other experiments it was substantial. For example, the Cd concentration in wheat grain at pH_{Ca} 4.0 and 5.0 in Experiment 6.5 (1989) (Fig. 6.2) was approximately one-and-a-half to two times the concentration in 1988 and 1987 (Fig. 6.2). This suggests that seasonal factors such as rainfall intensity and duration, and temperature may be involved. The effect of rainfall on root growth will depend on the amount and distribution of rain throughout the growing season. Since it was not possible to get rainfall data for each site, data from Rutherglen and Wagga Wagga were used as general approximations since Experiments 6.1-6.4 and 6.5-6.8 were in close proximity to these towns, respectively.

In 1987 and 1988 in Wagga Wagga, which were wet years (see Appendix 6.5), the Cd concentrations in grain were generally lower than in 1989. This could have been related to higher soil moisture in both 1987 and 1988 at the time of sowing and early crop growth (May-June) resulting in more rapid root growth which extended deeper into the soil profile and away from the region of higher Cd concentration ($\cong$ 0-10 cm).

Fig. 6.1.

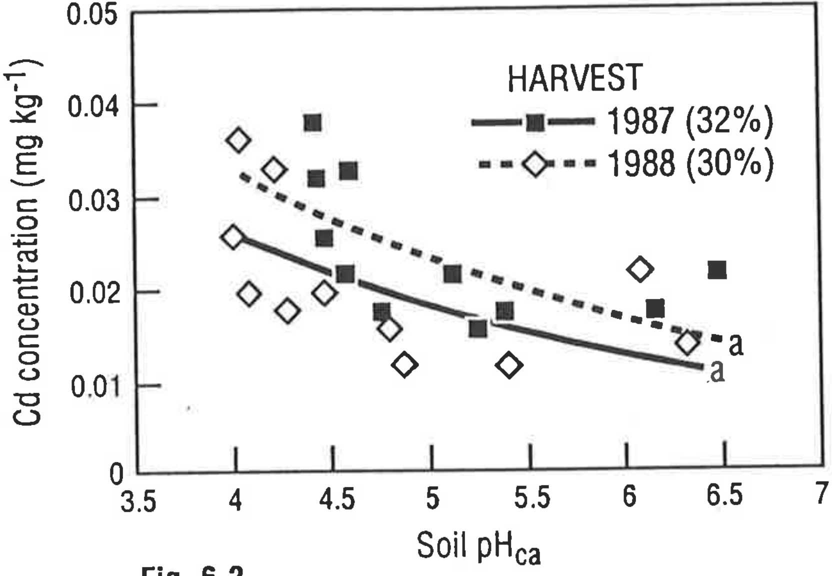
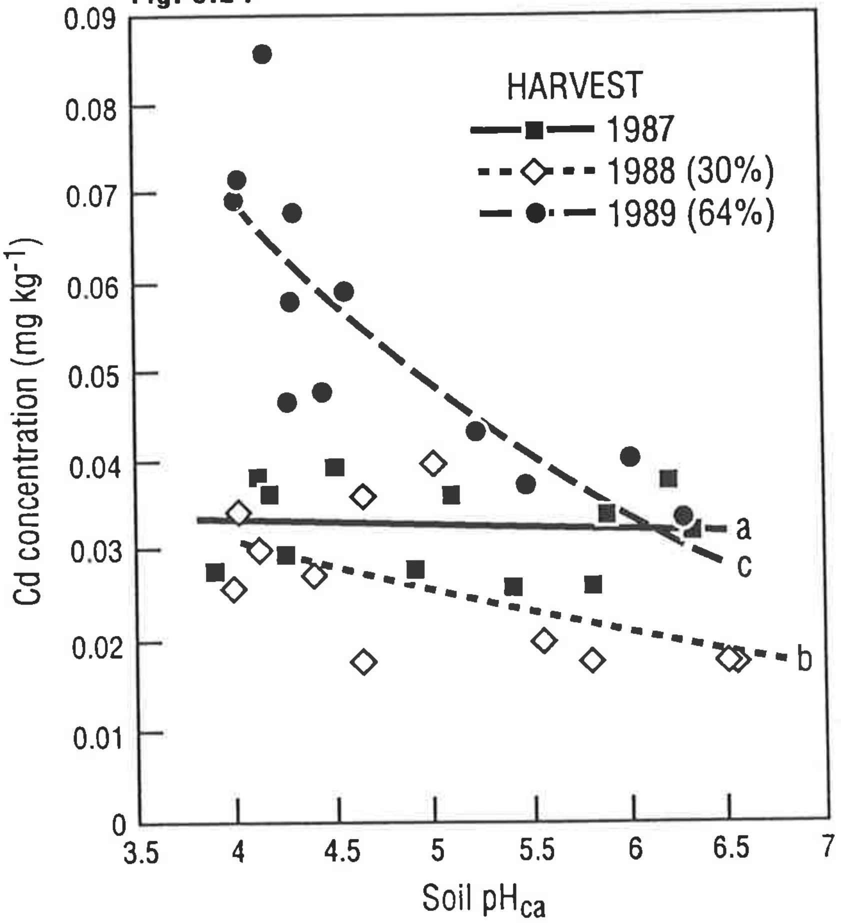


Fig. 6.2 .



Figures 6.1 and 6.2. Variation in grain Cd concentration (mg kg^{-1}) with soil pH for Experiments 6.2 (Fig. 6.1) and 6.5 (Fig. 6.2). All data are presented with the exponential model. In Figs. 6.1-6.4 statistical differences between years are distinguished by letters and the percent variance accounted for by the exponential model is given in brackets. When the data did not fit the model, no value is given.

Fig. 6.3.

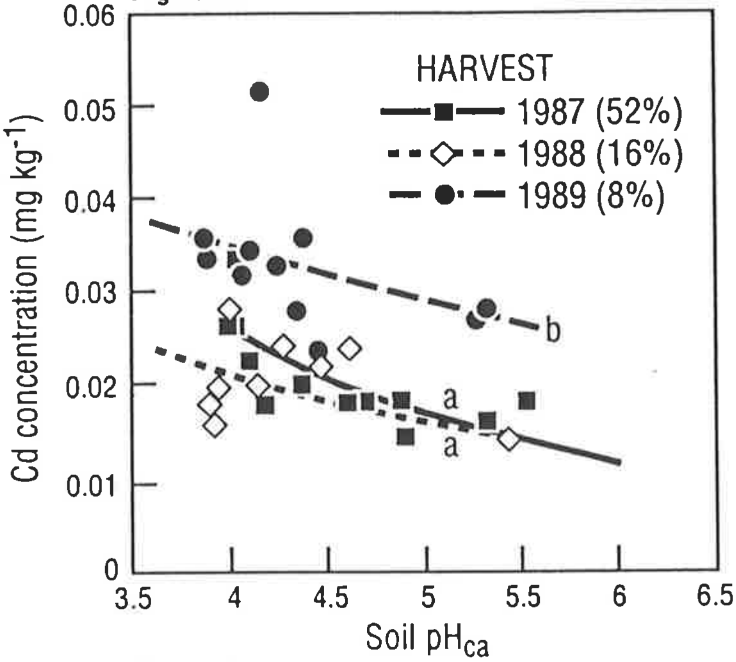
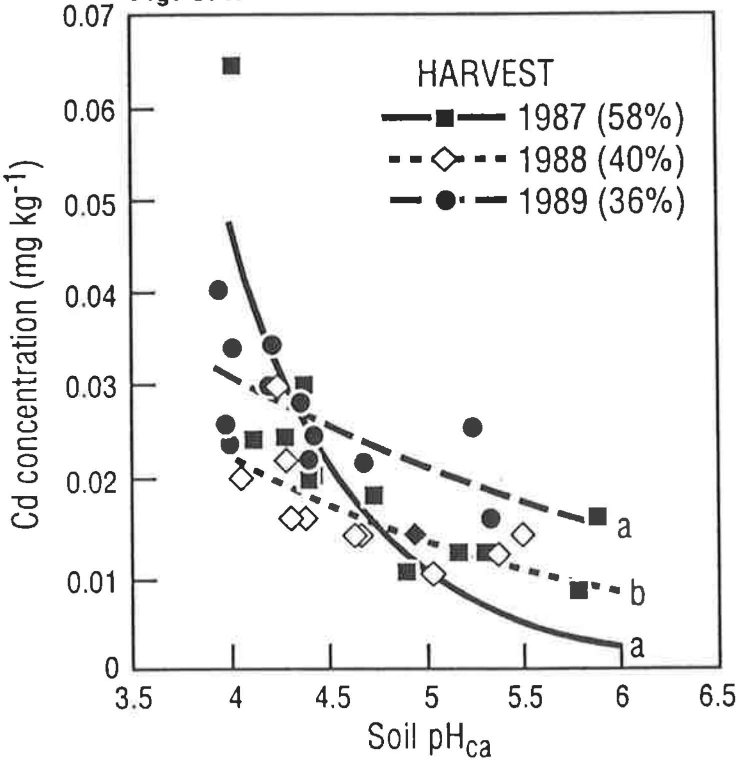


Fig. 6.4.



Figures 6.3 and 6.4. Variation in grain Cd concentration (mg kg^{-1}) with soil pH for Experiments 6.6 (Fig. 6.3) and 6.7 (Fig. 6.4). All data are presented with the exponential model.

D.Oliver, figures 6.3 and 6.4. D97/GER.

Although no temperature data are available from these sites it is known that temperature can also influence Cd uptake (Haghiri, 1974; Giordano *et al.*, 1979; and Sheaffer *et al.*, 1979). At lower soil temperatures lower concentrations of Zn and Cd have been found in broccoli and potato (Giordano et al., 1979) and maize grain (Sheaffer *et al.*, 1979).

Variation between sites

When the soil pH is similar the variation in Cd uptake by crops at different sites can often be explained by numerous soil and agronomic factors including variations in organic matter content (Haghiri, 1974), clay content (Eriksson, 1989), Fe- and Mn-oxide content and Cd concentration in the soil. Both clay and organic matter play important roles in retaining soil Cd through its many bonding sites which may render Cd less available to plants (Haghiri, 1974). Kuo *et al.* (1985) studied the effects of various soil characteristics on Cd and Zn availability to Swiss chard. They used soils with organic matter contents ranging from 1.6-6.9 % and found soil organic matter had little relation to metal availability.

The variation in grain Cd concentration between sites could not be explained by total organic carbon, EDTA-extractable Cd or clay content. For example, although the total organic carbon content varied from 1.08% in Experiment 6.8 to 1.71% in Experiment 6.2 (Table 6.1) the Cd concentrations in grain were similar at the two sites. Also, in experiments where the EDTA-extractable Cd concentrations covered a similar range (Table 6.1) the Cd concentrations in grain varied substantially. Possibly the range of EDTA-extractable Cd concentrations is too narrow to use site differences in EDTA-extractable Cd to explain the variation between experiments.

6.4.2 Plant Uptake

Any decrease in metal concentration in plants needs to be interpreted in relation to yield response to ensure that the decrease is not due to a growth dilution effect. Since no information was available on the effects of pH on dry matter production it was only possible to consider growth dilution effects in terms of increased grain yield. Of those experiments for which Cd concentration in grain decreased significantly with increasing pH, only the following had significant grain yield increases with increases in soil pH: Experiments 6.2 (1987 and 1988), 6.5 (1988) and 6.7 (1989).

Plots of Cd uptake (mg ha^{-1}) against soil pH_{Ca} showed decreases with increasing pH only for Experiment 6.2 (1987 and 1988) (Fig. 6.5). This suggests that for this experiment the significant decrease in grain Cd concentration with increasing soil pH was not due to a dilution effect as a result of increased grain yield. However, there was no clear relationship between Cd uptake into the grain and soil pH_{Ca} in Experiment 6.5 (1988) (Fig. 6.6). A similar response was found in Experiment 6.7 (1989) (Appendix 6.6). This suggests that for these experiments the relative increases in yield with increasing soil pH were greater than or equal to the decreases in Cd concentration in grain with increasing soil pH. Hence the decreases in Cd concentration in grain with increasing pH in these cases involve a yield component and cannot be attributed only to the effects of soil pH on the affinity of Cd for binding sites on soil particles.

□

□6.5 CONCLUSIONS

□In this field study the Cd concentration in wheat grain generally decreased steeply with increasing soil pH_{Ca} from 4.0 to 5.0 while the decrease became less steep with further increases in pH_{Ca} up to 6.0. Although the effectiveness of raising soil pH_{Ca} to 5.0 to minimise Cd uptake by wheat varied between seasons and sites the Cd concentration in grain did not increase in any experiment with increasing pH. In conclusion, as found with the light to medium textured soils in the glasshouse (Chapter 5), liming may be a useful management tool for decreasing Cd uptake by wheat grain. The practicalities of raising soil pH_{Ca} to 5.0 as a management practice to minimise Cd concentration in wheat grain are discussed further in Chapter 8.

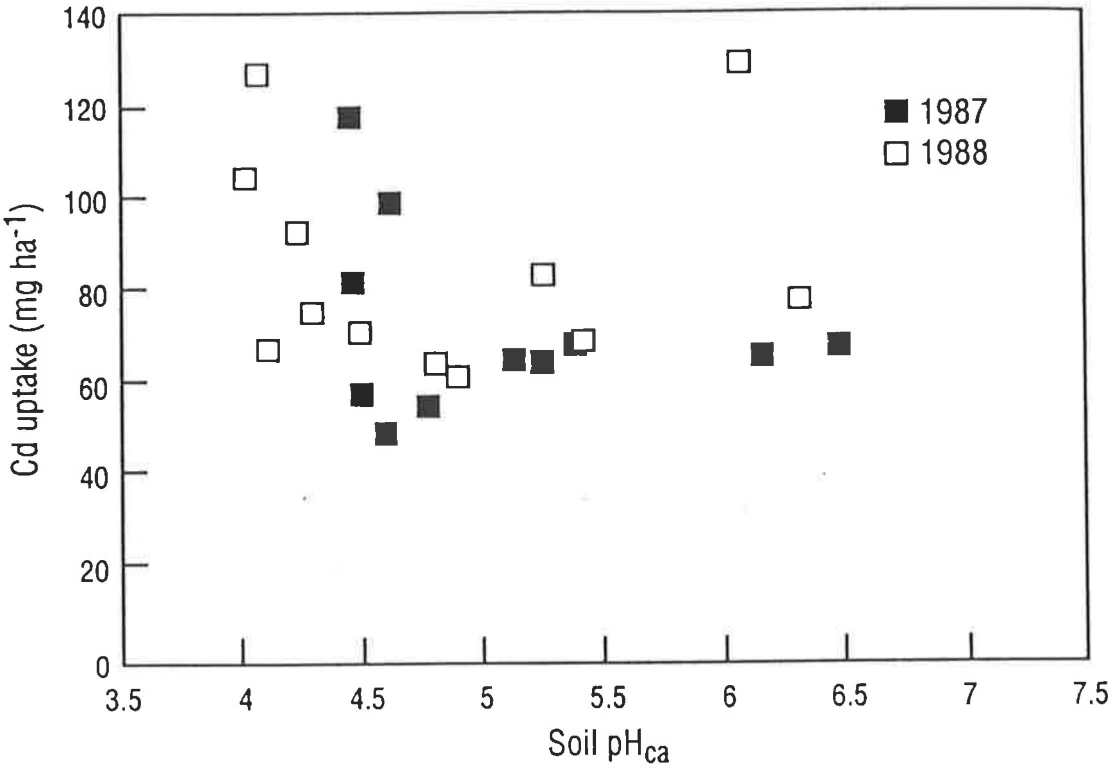


Figure 6.5. **Variations in Cd uptake (mg ha^{-1}) with pH for the 1987 and 1988 harvest of Experiment 6.2 at Rutherglen.**

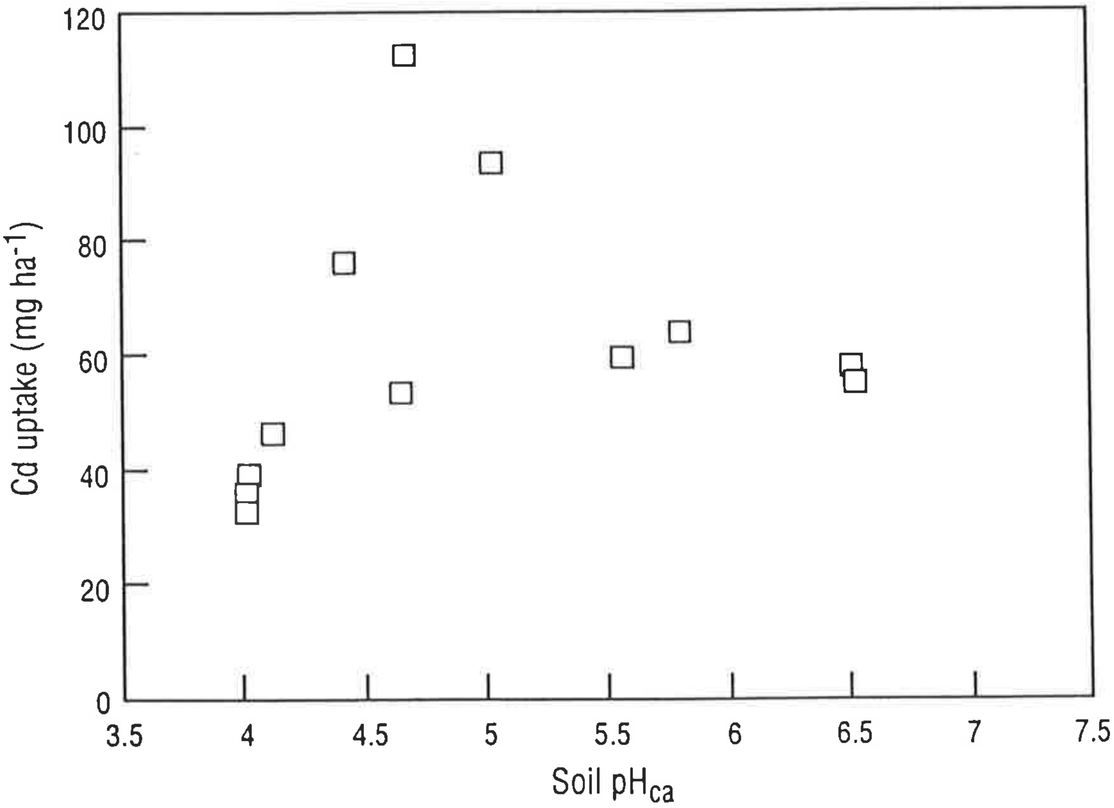


Figure 6.6. **Variations in Cd uptake (mg ha^{-1}) with pH for the 1988 harvest of Experiment 6.5 at Wagga Wagga.**

D.Oliver, figures 6.5 and 6.6. D98/GER.

CHAPTER 7

COMPARISON OF THREE SOIL EXTRACTION METHODS TO PREDICT Cd CONCENTRATION IN WHEAT GRAIN

7.1 INTRODUCTION

Chemical extractants provide a method for defining the concentrations of various fractions of metal ions in soils. In this study an effective soil test was defined as the chemical extraction that was simple, rapid and provided a good correlation with plant concentrations of the metal. Further the test must be applicable across a range of soil types and soil concentrations of the metal.

Many methods for determining plant-available Cd have been tested by researchers with varying degrees of success. For example, Symeonides and McRae (1977) found the best correlation between Cd concentration in radish tops and soil Cd concentration was obtained when 1N ammonium acetate-acetic acid buffered at pH 7.0 was used as the extractant. However, others (Street *et al.*, 1977; Whitten and Ritchie, 1991; Smilde *et al.*, 1992) have found better correlations between Cd concentrations in plant material and soil Cd concentrations using extractants that maintain the pH of the soil found in nature. In this study the effectiveness of three extractants to estimate plant-available Cd and predict Cd concentrations in grain was determined. The three extractants used in this study were: EDTA, $CaCl_2$ and $Ca(NO_3)_2$. These three extractants were chosen because they have been used by many other researchers but seldom compared and they extract different fractions of soil Cd.

7.2 MATERIALS AND METHODS

Soil samples from the glasshouse experiment (Chapter 5) and the rotation experiments (4.1 and 4.2) at Tarlee and Kapunda, respectively (Chapter 4) were used in this assessment. A brief outline of selected characteristics of the soils from the three experiments is given in Table 7.1.

Table 7.1 **Details of selected physical and chemical characteristics of soils used to assess the three soil extraction procedures**

	Soil Classification[1]	Soil Texture	% clay	% silt	% sand	pH$_{Ca}$ range[2]	%C range[2]
Glasshouse experiment soils							
Inman Valley	Sodic Haploxerert	clay	46	18	26	4.12-5.96	4.9
Kapinnie	Mollic Palexeralf	sandy loam	15	32	53	4.12-5.51	1.6
Freeling	Typic Rhodoxeralf	loamy sand/ sandy loam	9	10	79	4.22-5.85	1.2
Bordertown	Natric Palexeralf	loamy sand	8	5	87	3.92-6.09	1.5
Rotation experiment soils							
Tarlee	Calcic Palexeralf	sandy loam	14	13	72	1986: 4.68-7.35 1987: 4.53-7.40	0.82-1.25[3]
Kapunda	Calcic Natrixeralf	sandy loam	11	21	69	4.65-5.10	1.3-1.9*

[1] Soil Survey Staff, (1990).

[2] Range across pots or plots in each experiment. All measurements on the soils from the rotation experiments have been made on 0-10cm except those marked * which are 0-5cm.

[3] Mean values for 1986 and 1987.

Soil from two replicates from the glasshouse experiment (Chapter 5) and soil (0-10 cm) from plots that had received the extreme nitrogen treatments (N0 and N80) and been sown to selected crops (wheat/wheat; wheat/barley; wheat/lupins; and wheat/volunteer pasture) from Experiment 4.1 (Tarlee) was extracted with EDTA and $Ca(NO_3)_2$ as detailed in Chapters 3 and 4, respectively. Soil (0-5 cm) from all plots in Experiment 4.2 (Kapunda) were also extracted with EDTA and $Ca(NO_3)_2$. Soil from the glasshouse experiment which had not received any Cd treatment (Cd 0) was also extracted with $CaCl_2$ as detailed in Chapter 5 to allow comparisons to be made between the $Ca(NO_3)_2$ and $CaCl_2$ extraction procedures. An outline of the three extraction procedures is given in Table 7.2.

7.3 RESULTS

Results from analyses of variance of the effects of the treatments on the concentration of Cd extracted with EDTA, $Ca(NO_3)_2$ and $CaCl_2$ are given in the respective chapters. Details of the relationship between the Cd concentration in grain and the concentration of Cd extracted by each method are given in this chapter.

Glasshouse soil (Chapter 5)

The rate of change in grain Cd concentration (mg kg^{-1}) with increasing concentrations of EDTA-Cd, $Ca(NO_3)_2$ - Cd and $CaCl_2$ - Cd (for Cd 0 only) (mg kg^{-1}) and the corresponding regression coefficients (r^2) for the combined data from the glasshouse experiment and data for each individual soil in the glasshouse experiment are given in Table 7.3 and Figs. 7.1 and 7.3 for all Cd treatments and Table 7.4 and Figs. 7.2, 7.4 and 7.5 for Cd 0 only.

The relationship between the Cd concentration in grain and EDTA-extractable Cd was highly significant ($P<0.001$) for all four soils used in the glasshouse experiment across the range of Cd treatments applied (0-2.7 mg kg^{-1}) (Table 7.3 and Fig. 7.1).

Table 7.2 Outline of the extraction procedures used in this study

Extraction Procedure	Soil:Solution Ratio	Shaking Time	pH	Centrifuging Time and Speed	Specific Comments
0.05M EDTA[1,2]	1:2.5	7 days	6	4,000 rpm for 10 mins 16,000 rpm for 10 mins and filter then 6000 rpm for 20 mins.	preliminary manual shake; allow to stand for 2 hours to allow CO_2 to evolve; then put samples on shaker
0.01M $Ca(NO_3)_2$	1:5	4 hours	-	4,000 rpm for 15 mins.	-
0.01M $CaCl_2$	1:5	4 hours	-	4,000 rpm for 15 mins.	-

[1] ethylene diamine tetra-acetic acid
[2] Clayton and Tiller (1979)

Since Cd had been added to the soils in the glasshouse experiment the soil Cd concentrations were elevated and greatly exceeded the concentrations one would expect in Australian agricultural soils. Therefore grain Cd concentration data from all four soils to which no Cd had been added (Cd 0) were regressed against the Cd concentration extracted from the soil by all three methods. Repeating the regression analysis between the Cd concentration in grain and EDTA-extractable Cd for only soils to which no Cd had been added showed no significant relationship (Table 7.3 and Fig. 7.2).

As with the EDTA extractions, the relationship between the Cd concentration in grain and the concentration extracted from all four soils with $Ca(NO_3)_2$ was highly significant ($P<0.001$) for all Cd treatments applied (Table 7.4 and Fig. 7.3). However, when only data for soils to which no Cd had been added was considered the relationship between $Ca(NO_3)_2$ - Cd and Cd concentration in grain was highly significant ($P<0.001$) for the Kapinnie and Bordertown soils and significant ($P<0.05$) for the Freeling soil (Table 7.4 and Fig. 7.4). The relationship between $Ca(NO_3)_2$ - Cd and Cd concentration in grain was not significant for the Inman Valley soil ($r^2 = 0$) (Table 7.4).

The relationship between grain Cd concentration and $CaCl_2$ -Cd for the four soils used in the glasshouse experiment without any addition of Cd (Cd 0) was highly significant ($r^2=0.57$; $P<0.001$). The regressions for the Kapinnie, Freeling and Bordertown soils were significant ($r^2=0.65$, 0.45 and 0.78, respectively) (Table 7.4 and Fig. 7.5).

Table 7.3 Regression equations, coefficients and significance for the relationship between grain Cd concentration and EDTA- extractable Cd for soils from the glasshouse experiment which had received all Cd treatments (Cd0 to Cd2.7) and soils to which no Cd had been added (Cd0 only).

Data	y-intercept	Gradient	r^2	Significance
All Cd treatments (Cd0-Cd 2.7)				
Inman Valley	-0.024	+0.46	0.94	***
Kapinnie	-0.001	+0.95	0.75	***
Freeling	+0.016	+1.10	0.86	***
Bordertown	+0.102	+0.90	0.68	***
All Cd treatments for all soils	0.128	+0.61	0.64	***
Cd0 only				
Inman Valley	0.015	+0.365	0.12	NS
Kapinnie	-0.157	+2.37	0.22	NS
Freeling	0.303	-2.27	0.28	NS
Bordertown	0.041	+1.52	0.10	NS
All Cd0 data	0.133	-0.174	0.03	NS

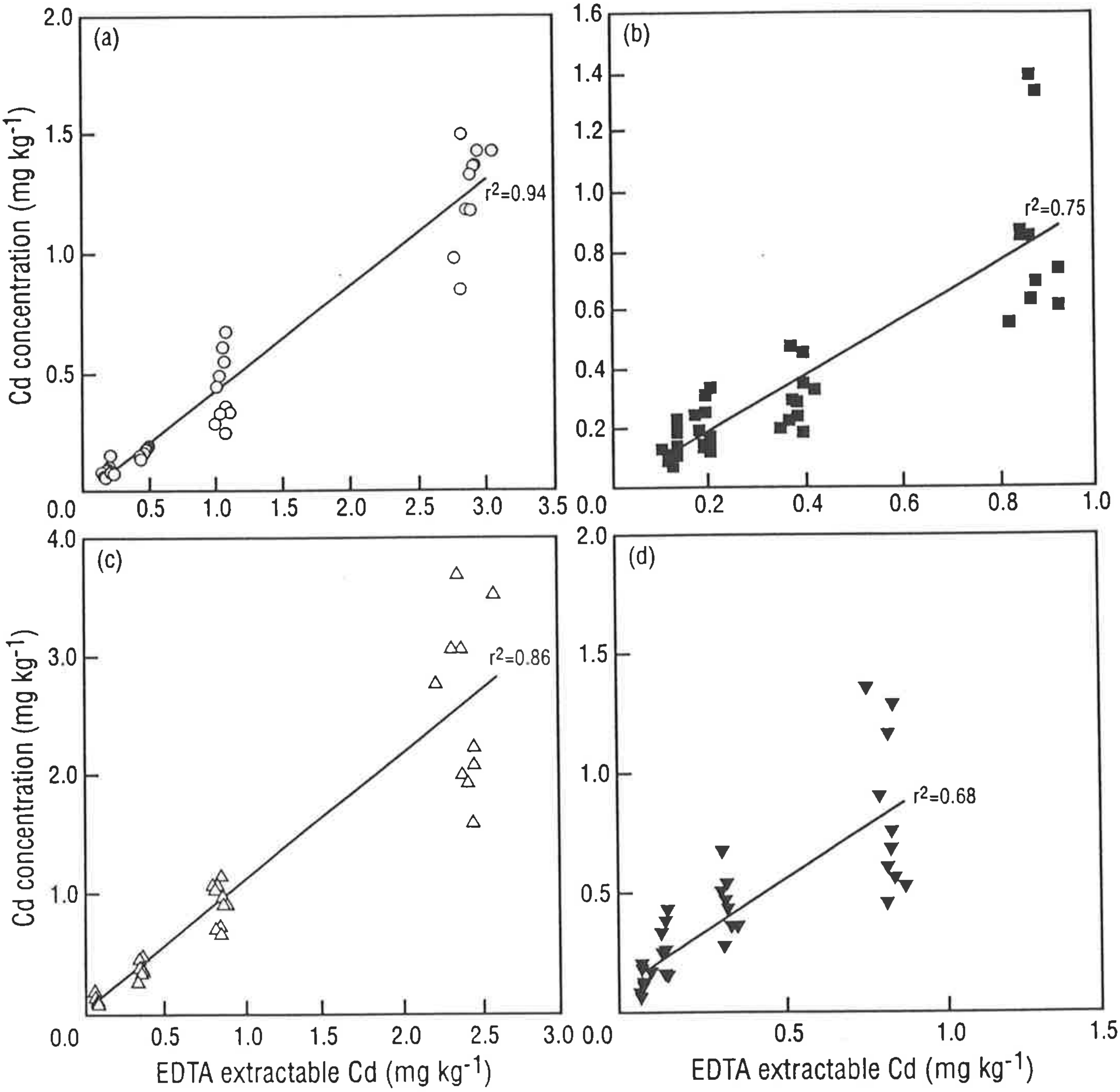


Figure 7.1. **Relationship between Cd concentration in grain and EDTA extractable Cd for the four soils used in the glasshouse experiment (Chapter 5) across all Cd treatments applied to the soil. The four soils are (a) Inman Valley; (b) Kapinnie; (c) Freeling and (d) Bordertown.**

D.Oliver, figure 7.1. D99/GER.

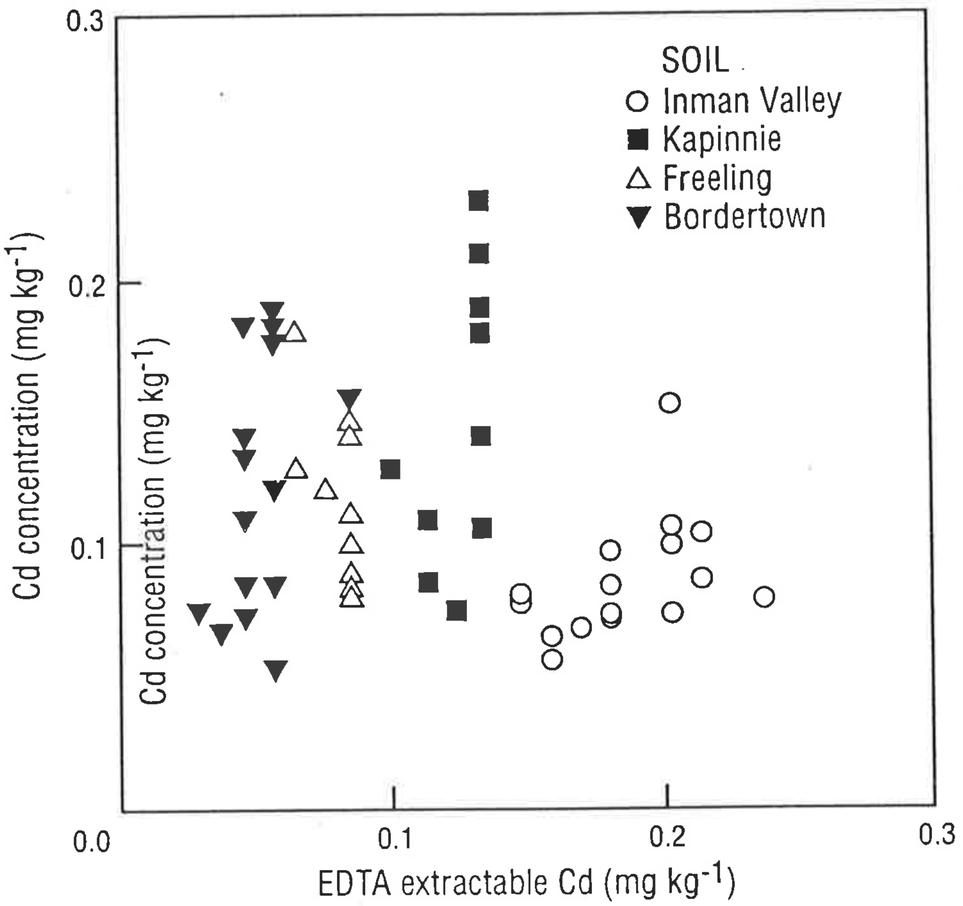


Figure 7.2. **Relationship between Cd concentration in grain and EDTA extractable soil Cd for the four soils used in the glasshouse experiment (Chapter 5) to which no Cd had been added (Cd0).**

D.Oliver, figure 7.2. D99/GER.

Table 7.4 Regression equations, coefficients and significance for the relationship between grain Cd concentration and $Ca(NO_3)_2$ - and $CaCl_2$ - extractable Cd for soils from the glasshouse experiment which had received all Cd treatments (Cd0 to Cd2.7) and soils to which no Cd had been added (Cd0).

Data	y-Intercept	Gradient	r^2	Significance
<u>All Cd treatments (Cd0-Cd 2.7)</u>				
$Ca(NO_3)_2$ - extractable Cd				
Inman[1] Valley	0.201	+9.45	0.32	***
Kapinnie	0.111	+4.67	0.75	***
Freeling	0.251	+3.78	0.80	***
Bordertown	0.162	+3.69	0.91	***
All Cd treatments for all soils	0.227	+3.75	0.72	***
<u>Cd0 only</u>				
$Ca(NO_3)_2$ - extractable Cd				
Inman Valley	0.085	+0.23	0	NS
Kapinnie	0.050	+5.26	0.70	***
Freeling	0.072	+2.69	0.39	*
Bordertown	0.041	+7.26	0.90	***
All Cd0 data	0.065	+3.99	0.55	***
$CaCl_2$ - extractable Cd				
Inman Valley	0.085	+0.14	0	NS
Kapinnie	0.051	+3.32	0.65	**
Freeling	0.063	+2.23	0.45	*
Bordertown	0.031	+5.10	0.78	***
All Cd0 data	0.059	+2.88	0.57	***

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[1] Two extreme values were removed

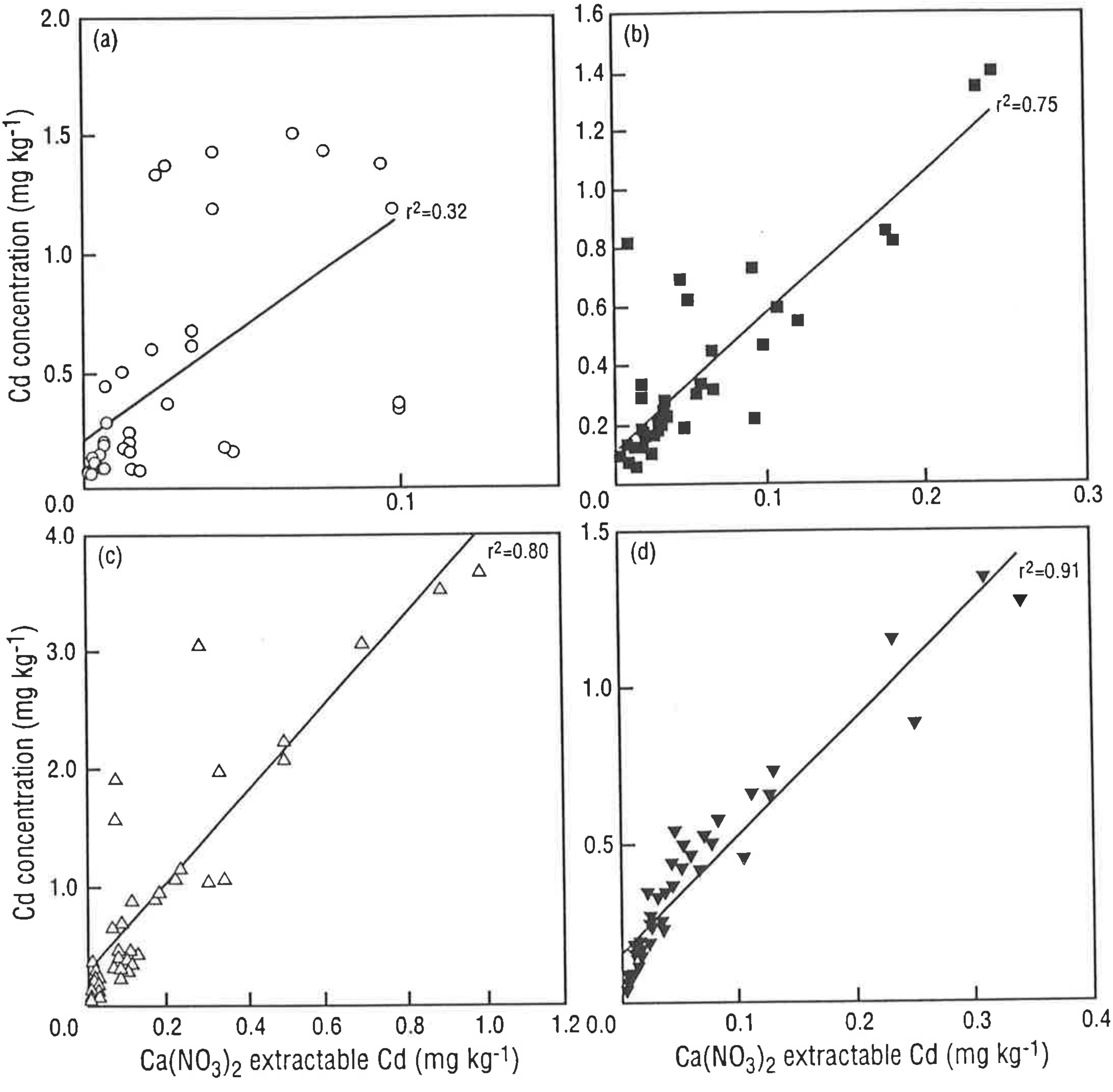

Figure 7.3. Relationship between Cd concentration in grain and Ca(NO$_3$)$_2$ extractable Cd for the four soils used in the glasshouse experiment (Chapter 5) across all Cd treatments applied to the soil. The four soils are (a) Inman Valley; (b) Kapinnie; (c) Freeling and (d) Bordertown.

D.Oliver, figure 7.3. D99/GER.

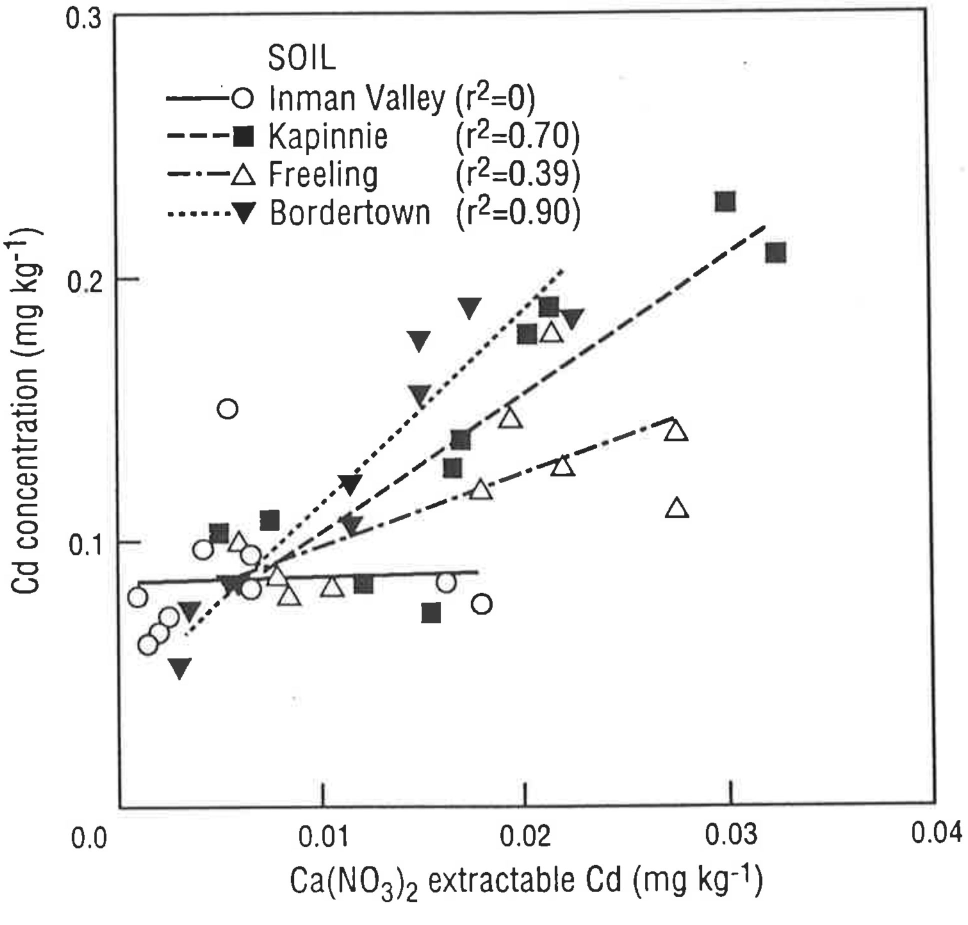

Figure 7.4. **Relationship between Cd concentration in grain and $Ca(NO_3)_2$ extractable Cd for all soils used in the glasshouse experiment (Chapter 5) to which no Cd was added (Cd0).**

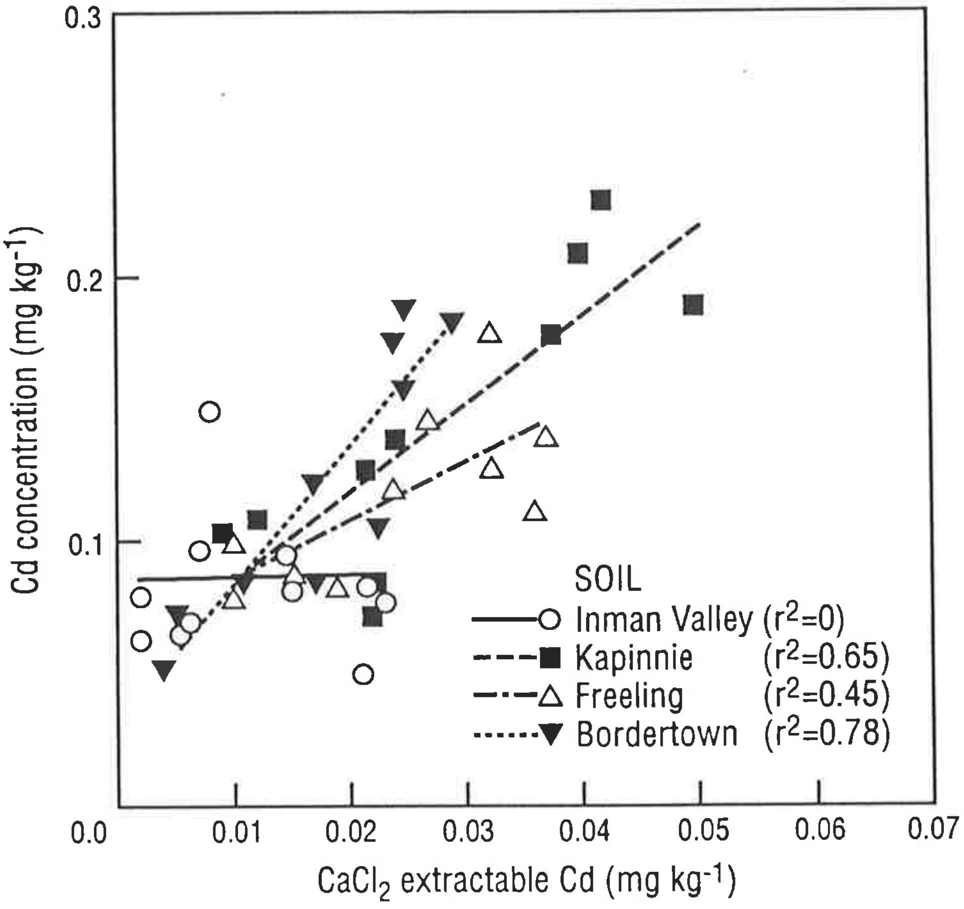

Figure 7.5. **Relationship between Cd concentration in grain and $CaCl_2$ extractable Cd for all soils used in the glasshouse experiment (Chapter 5) to which no Cd was added (Cd0).**

D.Oliver, figure 7.5. D99/GER.

Experiment 4.1 (Tarlee)

The regression of Cd concentrations in grain and EDTA-extractable Cd was significant only for the wheat/barley rotation ($P<0.01$) of the 1986 harvest of Experiment 4.1 (Table 7.5 and Fig. 7.6) and for the wheat/lupins rotation ($P<0.05$) of the 1987 harvest of Experiment 4.1 (Table 7.5 and Fig. 7.7).

Although the regression of grain Cd concentration on $Ca(NO_3)_2$ was significant ($r^2=0.12$; $P<0.01$) in 1986 (Table 7.6), the data were widely scattered (Fig. 7.8). None of the regressions for the individual rotations were significant (Table 7.6). Regression of the data from the combined rotations from the 1987 Tarlee experiment was highly significant ($r^2=0.25$; $P<0.001$). However, when the data were separated into individual rotations only the relationship from the wheat/lupin and wheat/barley rotations of this experiment were significant ($P<0.01$) (Table 7.6 and Fig. 7.9).

Experiment 4.2 (Kapunda)

The regression of Cd concentration in grain and EDTA-extractable Cd was not significant in Experiment 4.2 (Kapunda) either for the combined data or for the data separated into the three rotations (wheat/wheat, wheat/pasture and wheat/lupins) (Table 7.5).

The regression of Cd concentration in grain and $Ca(NO_3)_2$ - Cd for all the data from Experiment 4.2 (1990) was significant ($r^2=0.40$; $P<0.001$). When the data were separated on the basis of crop rotation only the regression of Cd concentration in grain and $Ca(NO_3)_2$ - Cd from the wheat/wheat rotation was significant ($r^2=0.26$; $P<0.05$) (Table 7.6 and Fig. 7.10).

Table 7.5 Regression equations, coefficients and significance for the relationship between Cd concentration in grain and EDTA-extractable Cd for all the data and data separated into individual crop rotations for the 1986 and 1987 harvests of Experiment 4.1 (Tarlee) and the 1990 harvest of Experiment 4.2 (Kapunda)

Data	y-intercept	Gradient	r2	Significance
1986 Tarlee				
wheat/wheat	0.160	-0.685	0.11	NS
wheat/barley	-0.039	+0.674	0.49	**
wheat/lupins	0.080	+0.149	0	NS
wheat/volunteer pasture	0.040	+0.130	0	NS
all data	0.073	-0.028	0	NS
1987 Tarlee				
wheat/wheat	0.091	-0.187	0	NS
wheat/barley	0.074	-0.172	0	NS
wheat/lupins	0.211	-0.874	0.34	*
wheat/volunteer pasture	0.054	+0.013	0	NS
all data	0.076	-0.081	0	NS
1990 Kapunda				
wheat/wheat	0.026	-0.026	0	NS
wheat/sown pasture	0.024	+0.017	0	NS
wheat/lupins	0.056	-0.145	0.13	NS
all data	0.030	+0.005	0	NS

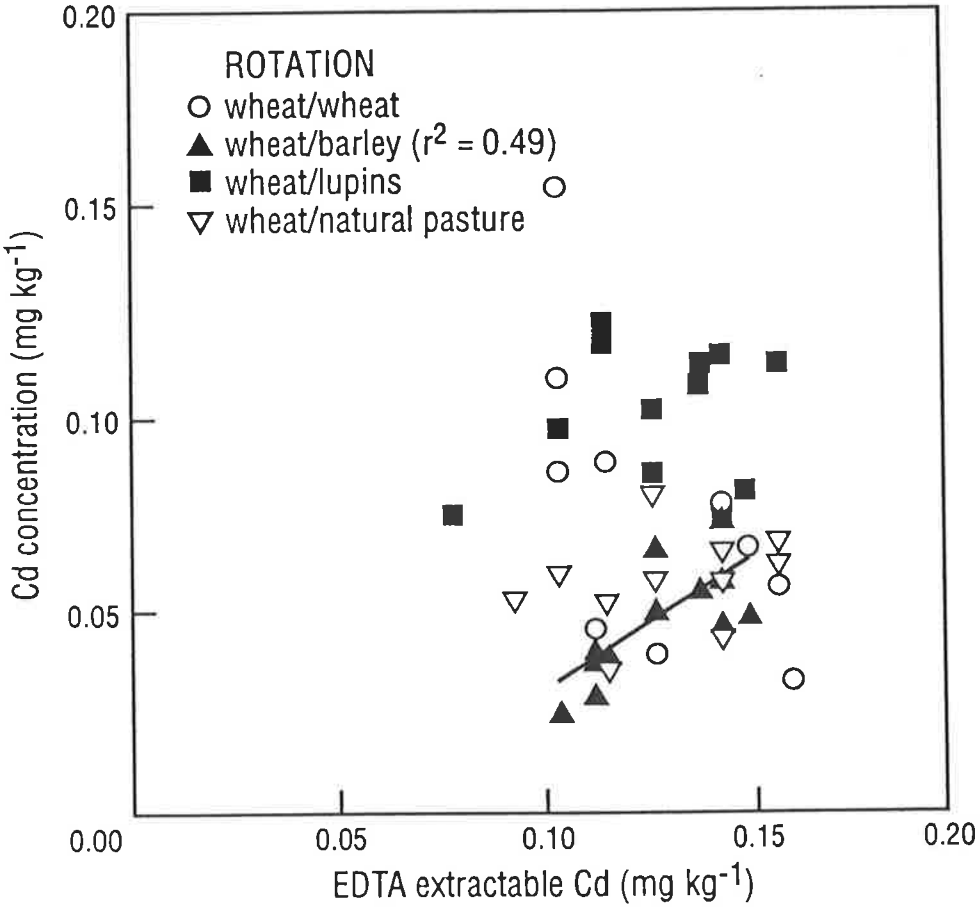

Figure 7.6. Relationship between Cd concentration in grain and EDTA extractable Cd from soil from Experiment 4.1 (1986). Only soil from selected rotations which had received the extreme nitrogen treatments (N0 and N80) was used in this assessment for Experiments 4.1 and 4.2.

D.Oliver, figure 7.6. D99/GER.

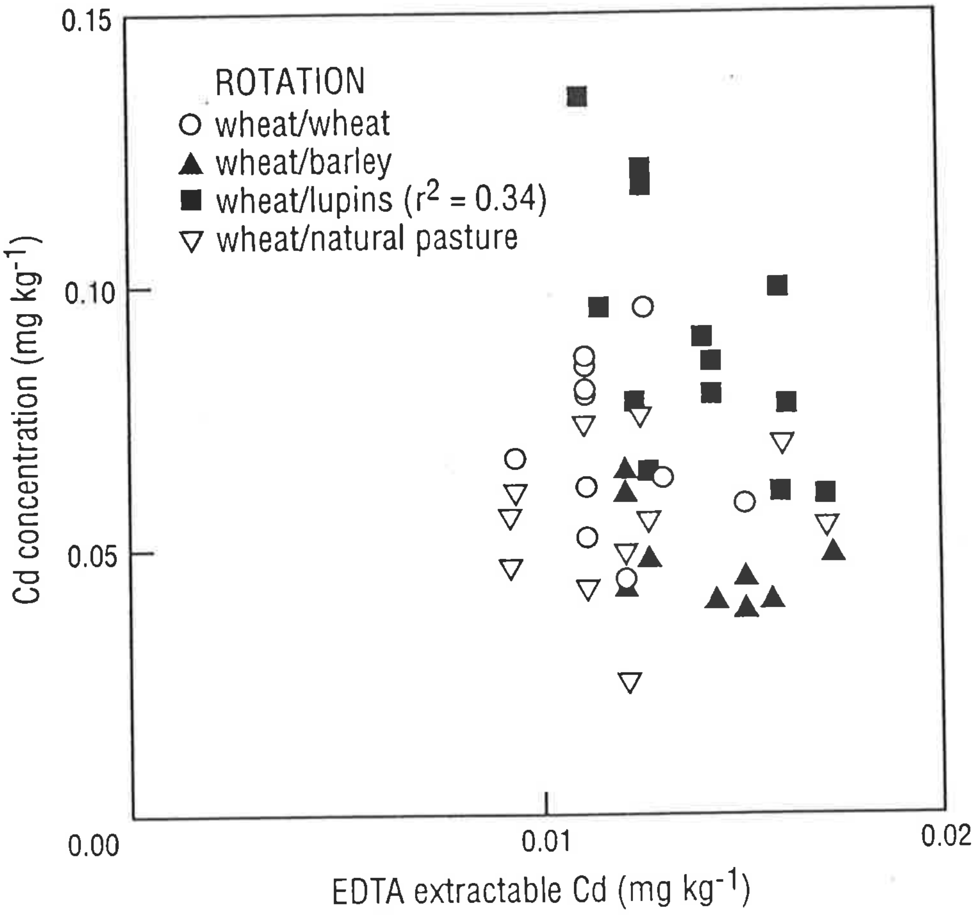

Figure 7.7. Relationship between Cd concentration in grain and EDTA extractable Cd for soils from Experiment 4.1 (1987).

D.Oliver, figure 7.7. D99/GER.

Table 7.6 **Regression equations, coefficients and significance for the relationship between grain Cd concentration and $Ca(NO_3)_2$ - extractable cadmium for all the data and data separated into the individual crop rotations for the 1986 & 1987 harvests of Experiment 4.1 (Tarlee) and the 1990 harvest of Experiment 4.2 (Kapunda)**

Data	**y-intercept**	**Gradient**	r^2	**Significance**
1986 Tarlee				
wheat/wheat	0.0671	+0.96	0	NS
wheat/barley	0.0443	+0.97	0	NS
wheat/lupins	0.0915	+0.91	0	NS
wheat/volunteer pasture	0.0541	+0.72	0	NS
all data[1]	0.0543	+2.75	0.12	**
1987 Tarlee				
wheat/wheat	.0588	+1.09	0.14	NS
wheat/barley	.0395	+1.71	0.43	*
wheat/lupins	.0406	+5.37	0.63	**
wheat/volunteer pasture	.0634	-1.85	0	NS
all data[1]	.0473	+2.39	0.25	***
1990 Kapunda				
wheat/wheat	.0046	+1.61	0.26	*
wheat/sown pasture	.0173	+0.68	0.11	NS
wheat/lupins	.0425	-0.27	0	NS
all data[1]	.0510	-2.08	0.40	***

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[1] Soil from only four rotations (wheat/wheat; wheat/barley; wheat/lupins; wheat/volunteer pasture) were extracted with $Ca(NO_3)_2$

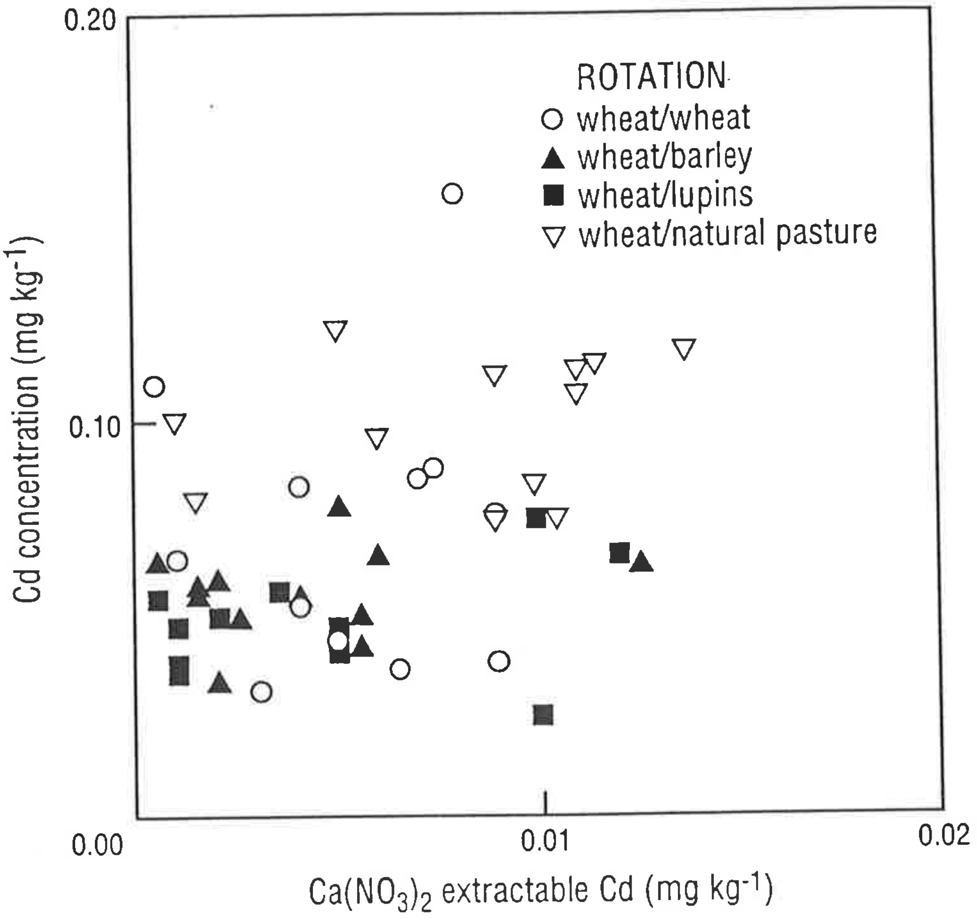


Figure 7.8. Relationship between Cd concentration in grain and $Ca(NO_3)_2$ extractable Cd for soils from Experiment 4.1 (1986).

D.Oliver, figure 7.8. D99/GER.

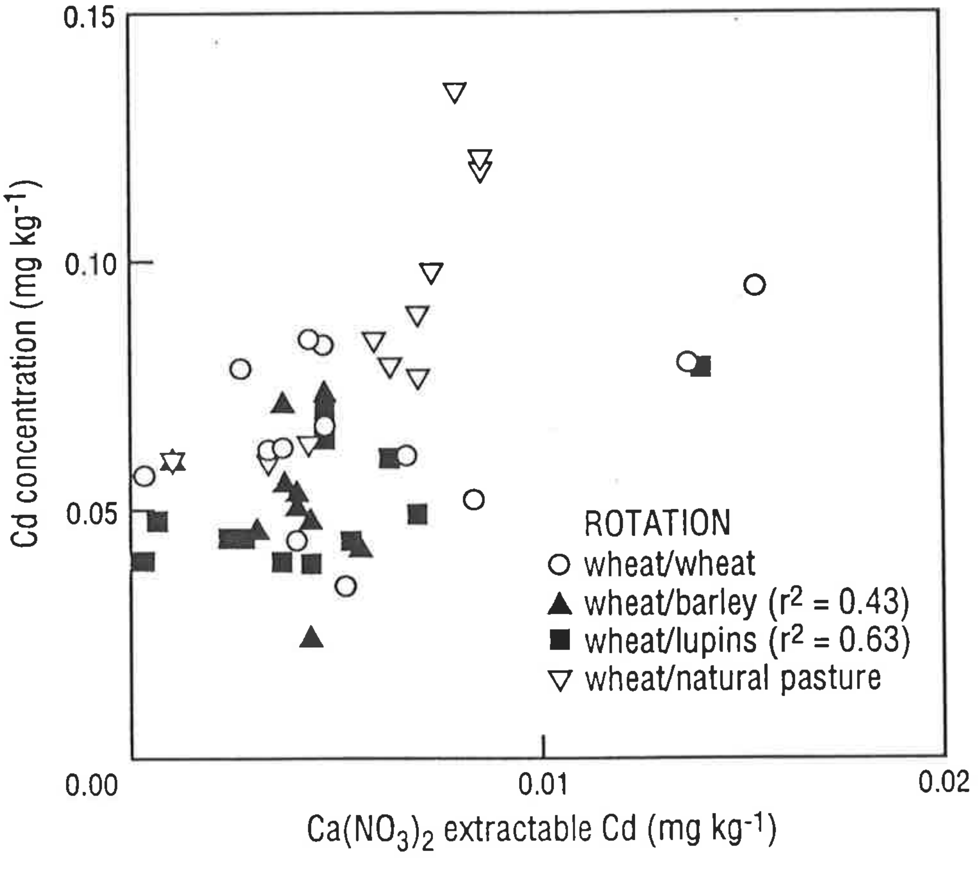


Figure 7.9. Relationship between Cd concentration in grain (mg kg^{-1}) and Ca(NO$_3$)$_2$ extractable Cd for soils from Experiment 4.1 (1987).

D.Oliver, figure 7.9. D99/GER.

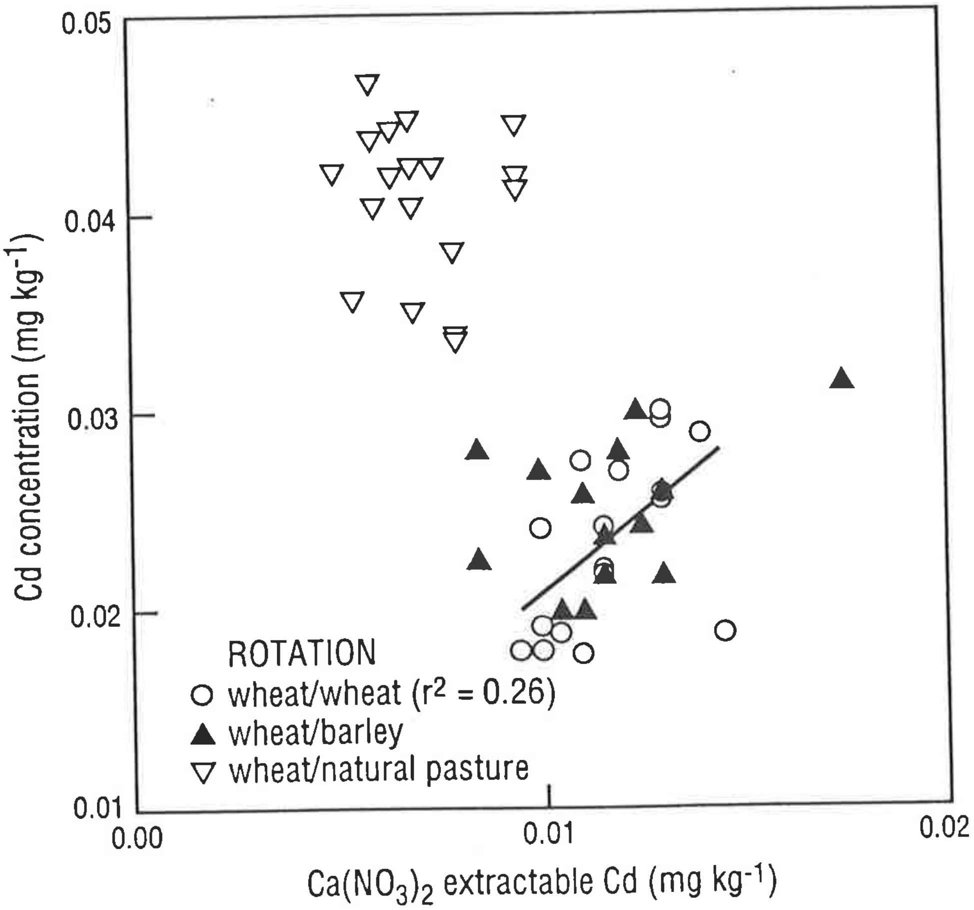


Figure 7.10. **Relationship between Cd concentration in grain (mg kg^{-1}) and Ca(NO$_3$)$_2$ extractable Cd for soils for three rotations in Experiment 4.2 (1990) at Kapunda.**

7.4 DISCUSSION

EDTA extractions

Generally there was a poor relationship between EDTA-extractable Cd and Cd concentrations in grain. The only exception to this was the regression between Cd concentration in grain and EDTA-extractable Cd for each individual soil used in the glasshouse experiment (Chapter 5) when all Cd treatments were considered (Table 7.3). However, the significant ($P<0.001$) correlation was due to the wide range of soil Cd concentrations covered. Symeonides and McRae (1977) also found the Cd concentration in radish tops grown on soils to which Cd had been added was poorly correlated with EDTA (0.05N) extractable Cd.

Merry and Tiller (1978) however, found good correlations between Cd concentrations in plant material of several species that were grown in the field and EDTA-extractable Cd. However, in their study all soils were similar with pH_W values generally greater than 8. A good relationship between EDTA-extractable Cd and Cd concentration in grain would also be expected for soils with lower pH provided the main soil characteristics that affect Cd availability for plant uptake (viz. soil pH, organic carbon content, and clay content) were constant. Further, the soils used in the studies of Merry and Tiller (1978) had higher concentrations of Cd than those in this study due to the close proximity of the sampling sites to a Pb-Zn smelter.

In the glasshouse experiment (Chapter 5) most of the Cd applied to the soils as $CdSO_4$ was extracted with EDTA despite equilibrating the soils by a wetting and drying process for several months. This suggests either that the processes involved in Cd adsorption to soil fractions take a very long time and/or that EDTA is unlikely to be a useful extractant for determining the Cd fraction available for plant uptake.

One problem associated with using EDTA extractions for predicting Cd concentrations in plant material or grain is that the EDTA solution is buffered at pH 6.0. Since uptake of plant Cd depends on its concentration in soil solution and on its release from soil colloids to the soil solution, both of which are affected by pH, the chemical extractant should ideally not alter the pH of the soil. Consequently many researchers (Bjerre and Schierup 1985; Sauerbeck and Styperek, 1985; Whitten and Ritchie, 1991; Smilde *et al.*, 1992) have suggested that dilute solutions of neutral salts are more suitable extractants for predicting Cd concentrations in grain. However, other studies have used only a small number of soil samples (Whitten and Ritchie, 1991; Smilde *et al.*, 1992) and/or used soils which have very similar physical and chemical characteristics (Whitten and Ritchie, 1991).

$CaCl_2$ and $Ca(NO_3)_2$ extractions

In this study there was a good relationship between $Ca(NO_3)_2$-extractable Cd and Cd concentration in grain from plants grown on soil to which Cd had been added and on soil to which no Cd had been added for two soils in the glasshouse experiment : Kapinnie and Bordertown (Figs. 7.3 and 7.4). Although the correlation between Cd concentration in grain and $Ca(NO_3)_2$ - extractable Cd was good for plants grown on the Freeling soil when all data was considered (Table 7.4 and Fig. 7.3), $Ca(NO_3)_2$ - extractable Cd accounted for only 39% of the variation in Cd concentration in grain when only data from Cd 0 was considered (Table 7.4 and Fig. 7.4). The correlation was poor for plants grown on the soil with the highest clay content : Inman Valley. The correlation was also generally poor for Experiments 4.1 and 4.2 (Table 7.6).

As with the $Ca(NO_3)_2$ extractions, the best correlations between Cd concentration in grain and $CaCl_2$ - Cd was with plants grown on the Kapinnie and Bordertown soils and the poorest correlation was with plants grown on the Inman Valley soil (Table 7.4). The most likely reason for the poor relationship between EDTA-, $Ca(NO_3)_2$ - and $CaCl_2$ - Cd and Cd concentration in grain from plants grown on the Inman Valley soil was the lack of response of extractable Cd concentrations to changes in pH of this soil (see Chapter 5).

Although Smilde *et al.* (1992) found a good correlation between Cd concentration in maize and soil Cd extracted by 0.1M $CaCl_2$ only two soils were used in their study and the data for the loamy soil was clustered at the origin. Similarly, Whitten and Ritchie (1991) found $CaCl_2$ extractions were a good indicator of Cd concentrations in whole tops of subterranean clover but only three soils were used in their study and all three soils were very sandy (>80% sand) with pH_{Ca} values ranging from only 4.4 to 4.8.

Chloride ions form stable complexes with Cd while Cd does not form complexes with nitrate. Therefore, one would expect $CaCl_2$ to extract a higher concentration of Cd than $Ca(NO_3)_2$. In this study, the slope of the regression of grain Cd concentration on extractable soil Cd was greater for the combined data on all soils and for each individual soil with $Ca(NO_3)_2$ than with $CaCl_2$ as the extractant (Table 7.4). Therefore, for the same concentration of Cd in the grain more Cd was extracted from the soil using $CaCl_2$ than with $Ca(NO_3)_2$. However, provided the relationship has a strong correlation, the quantity of Cd extracted from the soils is not important and the soil test is still suitable for predicting Cd concentration in grain.

Both $CaCl_2$ and $Ca(NO_3)_2$ underestimated the actual concentration of soil Cd available for plant uptake. This is indicated by extrapolation of the regression lines for the glasshouse soils (Figs. 7.4 and 7.5) where the y-intercept does not pass through the origin. In other words, when no Cd is extracted from the soil using $CaCl_2$ or $Ca(NO_3)_2$ there are still measurable concentrations of Cd in the grain. This further indicates a limitation of the extraction procedures.

Although only four soils were used in the glasshouse experiment the physical characteristics of the soils used were quite varied and the pH_{Ca} of the soils covered a range of approximately 4.0-6.0 (Table 7.1). Ideally a larger number of soils ranging in physical and chemical characteristics should be used to assess the suitability of a soil test. However, in a glasshouse experiment the environmental conditions for plant growth are optimised and if a soil test does not correlate well with Cd concentrations in grain under these circumstances then it would be unlikely to do so in the field. The strongest correlation between grain Cd concentrations and $Ca(NO_3)_2$ - Cd (ie. the least scatter of data around the regression line) of soils from the glasshouse experiment was in the following order: Bordertown > Kapinnie = Freeling >> Inman Valley. The relationships for the two field experiments at Tarlee and Kapunda were similar. The poor correlation between Cd concentration and $Ca(NO_3)_2$ - Cd for grain from Experiments 4.1 and 4.2 (Table 7.6) may be due to the sampling technique. A subsample of grain and a subsample of a bulked sample of ten cores of soil across the plots were used in both experiments. Possibly the soil test may only be applicable when paired soil and grain samples are used.

7.5 SUMMARY AND CONCLUSIONS

In this study good correlations were found between Cd concentrations in grain grown on light textured soils and $CaCl_2$- and $Ca(NO_3)_2$-extractable soil Cd. However, $CaCl_2$ did extract a higher concentration of Cd than that extracted by $Ca(NO_3)_2$. When the plants were grown on heavier textured soils neither extractant provided a good correlation between grain Cd concentration and extractable Cd. The correlations between Cd concentration in grain and EDTA-extractable Cd were poor for all soil Cd concentrations that were representative of those found in Australian agricultural soils.

In the glasshouse experiment (Chapter 5) the ratio of Cd grain : Cd shoot was highly variable with many of the treatments applied which suggests a large variation in the distribution of Cd within the plants. Possibly soil tests may provide better correlations with Cd concentrations in whole tops rather than Cd concentrations in grain only.

In conclusion, better correlations between the soil extraction and Cd concentration in grain were found when unbuffered neutral salts were used as the extractant rather than

EDTA. However, the variable distribution of Cd within the plant may limit the usefulness of soil extractants for determining Cd concentrations in grain.

CHAPTER 8

SUMMARY AND CONCLUSIONS

A review of the literature indicated a large body of overseas research on Cd accumulation in plants, but little information about Cd concentrations in Australian food products. Furthermore, the data obtained overseas are not easily transferable to Australian conditions because the majority of investigations have involved high Cd concentrations in soil resulting either from the application of sewage sludge to soil or from atmospheric deposition from smelters.

The maximum permissible concentration (MPC) for Cd in Australian grain, which is set by the National Health and Medical Research Council (NHMRC), is 0.05 mg kg^{-1}. The Cd concentrations in Australian food are generally low and do not pose any health threat. The main concern about Cd concentrations in Australian grain is from a trade viewpoint. Where grain is blended from many sources before it is sold any problems with high (> 0.05 mg kg^{-1}) Cd concentrations in grain can be overcome by dilution during the blending process. However, when grain is exported from selected regions to speciality markets, the options for blending decrease. The policy of the Australian Wheat Board to ensure high quality products for the export market has necessitated a better understanding of Cd concentration in grain and the management factors that can be manipulated to minimize Cd uptake by grain.

This study investigated various management factors available to farmers to minimise Cd concentrations in wheat grain. The results of these investigations are summarised below and some indications are given of future work.

8.1 ZINC

Field investigations were conducted to determine the effects of soil-applied Zn, to ameliorate Zn deficiency, on Cd concentration in wheat grain. In 13 experiments at 9 Zn-deficient sites across South Australia, applications of Zn decreased Cd concentrations in grain. In some cases the Cd concentrations in grain were halved. Generally, at Zn rates greater than 2.5 - 5.0 kg Zn ha^{-1} no further decreases in Cd concentrations in grain were found. Although only one sample in this study exceeded the MPC, the application of Zn to the soil is potentially a very useful management technique for minimising Cd concentrations in grain. In South Australia, commercial $ZnSO_4$ for application to the soil retailed in 1993 at approximately at $A1.00 - $A1.50 L^{-1}. The commercial $ZnSO_4$ contains 0.167 kg Zn L^{-1} and the cost of applying 2.5 kg Zn ha^{-1} is approximately $A15.00 to $A22.50, which is an economically viable option for farmers to minimize Cd concentrations in grain in areas that are deficient or marginally deficient in Zn.

The mechanisms involved in the decrease in Cd concentration in grain with applications of Zn to soils containing inadequate Zn for wheat growth were not investigated but possible mechanisms are given below.

i. The increased concentration of Zn ions in soil solution may compete more strongly with Cd ions in soil solution at the root - soil solution interface and thus may be taken up by the plant in greater proportion than Cd ions.

ii. Under conditions of Zn deficiency, the selectivity of the root membrane of certain plants has been shown to be disrupted possibly resulting in greater Cd uptake. Applications of Zn to the soil to prevent Zn deficiency in the plant would prevent this occurring, which could result in a decrease in the concentration of Cd accumulated in the grain.

iii. Under conditions of Fe and Zn deficiency, plants have been found to produce phytosiderophores to "sequester" nutrients. Possibly, phytosiderophores which were produced by Zn deficient wheat plants in these experiments, increased uptake of Cd. This may have resulted in higher Cd concentration in grain than those in plants that were not Zn deficient.

These mechanisms could be investigated by studying the production of phytosiderophores and the selectivity of root membranes of wheat plants grown with different Zn and Cd concentrations in solution culture.

Since this study involved soil-applied Zn, additional information is required to determine whether the same response would be found when Zn is applied in foliar sprays. This would be extremely useful information in areas where the possibility of high Cd concentrations in grain was not realised until after the crop had been sown and seedlings had emerged. The effectiveness of applying Zn, either to the soil or as foliar sprays, to minimise Cd concentrations in wheat grain when the plants are not Zn deficient also requires additional studies.

Further investigations could also focus on the effects of amelioration of other nutrient deficiencies, such as Cu, Mn and Fe on Cd concentrations in wheat grain. However, the effectiveness of amelioration of these nutrient deficiencies to minimise Cd concentrations in grain will depend upon the physiological effect of each particular nutrient deficiency on the plant and/or whether these ions, upon addition to the soil, would compete for physical adsortion sites either at the root or soil surfaces.

8.2 CROP ROTATIONS, STUBBLE HANDLING PRACTICES AND TILLAGE METHODS

The three stubble handling practices (stubble burning, stubble incorporation and stubble retention), and the three tillage methods (conventional cultivation, direct drilling and reduced till) studied were found not to have any significant effect on Cd concentration in grain.

Crop rotation however, had highly significant ($P<0.001$) effects on grain Cd concentration, with the highest concentrations being found in wheat grain grown in the year following a lupin crop and the lowest Cd concentrations in wheat grain grown after barley.

Although the exclusion of lupins from rotation sequences is not advocated, it is important to consider the choice of wheat variety grown after a lupin crop and/or the sequence of crops in the rotations. Although wheat is commonly grown after lupins it may be possible to include other crops in the rotation sequence. On the southern Eyre Peninsula, South Australia, a possible alternative crop to include in rotation with wheat that is now being considered is canola. However, there is no published information about the impact of growing canola in the year before a wheat crop on Cd concentrations in wheat. Alternatively, in the higher rainfall areas durum wheat, (*Triticum durum*), may be grown rather than bread wheat (*T. aestivum*). Some durum wheats have been found to have much lower grain Cd concentration than that found in bread wheats, but work is required to assess Cd uptake by Australian durum wheat cultivars.

Further work is required to assess the reasons for the higher Cd concentrations in grain grown after a lupin or other leguminous crop and lower Cd concentrations in wheat grain grown after barley. This could include soil solution investigations, paired sampling of grain and soil for rhizosphere studies from crop rotation experiments, studies on Cd accumulation in the roots of different crops and the release of Cd during the decomposition of plant roots. In addition, the effects of crop rotation on Cd concentration in grain need to be investigated in a wider range of environmental conditions and soil types.

8.3 SOIL pH

The effects of pH on Cd concentration in grain were assessed under glasshouse conditions and in the field. While raising soil pH_{Ca} from 4.0 to 6.0 was an effective method for decreasing the Cd concentration in grain grown in the glasshouse experiment on light textured soils it was ineffective on the heavier textured soils. The

Cd concentration in grain grown on the heavier textured soil, a Sodic Haploxerert from Inman Valley, may have decreased had the pH_{Ca} been raised above 6.0, but the cost of applying lime to raise the soil pH_{Ca} above 6.0 and then maintaining the pH_{Ca} above this level would most likely make this option for minimising Cd concentrations in grain uneconomical.

Raising soil pH to minimise the Cd concentration in grain was generally more effective in the glasshouse experiment than in the field experiments, which is not an unexpected result in view of the more favourable and consistent environmental conditions provided for plant growth in the glasshouse.

The results from the field experiments were variable. While the Cd concentration in grain decreased at some sites with increases in pH, no response was seen at other sites. Further, responses varied from year to year at the same site. The inconsistent results from the field experiments illustrate the problems associated with extrapolating data from a glasshouse experiment to the field and the need to conduct field experiments to validate results found in glasshouse studies. The reasons for the large differences between years in Cd concentration in grain at one site were not clear and may relate to variation between years in rainfall distribution and frequency and its effect on root distribution in the soil profile or to variation in soil temperature. While raising soil pH to minimise Cd concentrations in grain is not always effective it is still a useful management tool which should be considered when considering options for minimising Cd concentrations in grain.

8.4 SOIL TESTS

The correlations between Cd concentrations in grain and extractable soil Cd using EDTA, $Ca(NO_3)_2$ and $CaCl_2$ were determined. The EDTA-extractable Cd did not correlate well with Cd concentrations in grain in either the field or glasshouse experiments. The lack of correlation is consistent with EDTA extractants measuring totally potentially available soil Cd. Good correlations between Cd concentrations in grain and $Ca(NO_3)_2$- and $CaCl_2$-extractable Cd were found for grain grown in the glasshouse study on light textured soils but not on the heavier textured soil (Inman Valley). However, poor correlations were found between Cd concentrations in grain grown in the field experiments and extractable soil Cd using either extractant. However, the poor correlation between $Ca(NO_3)_2$- and $CaCl_2$- extractable Cd and Cd concentration in grain may be a result of the soil sampling procedure used, environmental conditions, variation in the distribution of Cd between the grain and the rest of the shoot, and the limited range of soils used in the assessment. On the basis of this study the best correlations between Cd concentration in grain and extractable soil Cd were found using $Ca(NO_3)_2$ as the extractant, but further investigations are required to assess the effectiveness of $Ca(NO_3)_2$ and $CaCl_2$ for predicting Cd concentrations in grain across a wider range of soil types varying in both physical and chemical characteristics.

8.5 CONCLUSIONS

In conclusion, this study has identified the following factors that determine Cd uptake by wheat grain and which may be manipulated to minimise Cd uptake.

1. The Zn status of the plant influences Cd uptake. To minimize Cd uptake by wheat farmers should arrange sampling of the soil so that DTPA-extractable Zn concentration may be determined. If the soil has inadequate Zn for crop growth, even if only marginally inadequate, then Zn should be applied to the soil. In this study applications of 2.5-5.0 kg Zn ha^{-1} were found to decrease the Cd concentration in grain by up to one-half.

2. The sequence of crops grown in rotation influences Cd concentration in grain. Where wheat is grown continuously in rotation with lupins it may be necessary

to include an alternative crop prior to the wheat crop being grown or to grow wheat varieties that accumulate less Cd.

3. Although raising soil pH is the most commonly recommended practice for minimising Cd concentrations in grain this study showed that in the field this is not always an effective strategy. The variation between years in the response of Cd concentration in grain with increases in soil pH made it difficult to state under what conditions liming would be effective for minimising Cd uptake by grain. Further experiments are required to determine the reasons for annual variations in the response of Cd concentrations in grain to changes in soil pH.

4. Of the three soil extractants used in this study the best correlations between Cd concentration in grain and extractable soil Cd were found using $Ca(NO_3)_2$. However the correlations between extractable Cd and Cd in grain grown in the field indicate that there may be limited use for soil tests for predicting Cd concentration in grain.

APPENDICES

Appendix 3.1 Mean EDTA - extractable Cd (n=2) concentrations (mg kg^{-1}) for all Zn treatments of Experiments 3.1 and 3.3-3.13 in South Australia

Experiment	Zn Rate (kg ha^{-1})											
	0	0.1	0.25	0.5	1.0	2.5	5.0	7.5	10.0	15	20	s.e.d.
i *"Fresh" Zn*												
3.1	0.063	0.064	0.072	0.068	0.063	0.064	0.043	0.043	0.051	0.510	0.059	0.008
3.3	0.050	-	-	-	0.041	0.046	0.058	-	0.063	-	0.053	0.004
3.4	0.011	-	-	-	0.015	0.015	0.011	0.019	0.015	-	0.031	0.003
3.5	0.011	-	0.007	0.007	0.007	0	0.007	-	0.14	-	-	0.003
3.6	0.066	0.028	0.037	0.039	0.022	0.039	0.055	0.028	0.033	0.056	0.061	0.011
3.7	0.044	0.039	0.055	0.044	0.039	0.021	0.027	0.031	0.023	0.043	0.055	0.007
3.8	0.069	0.064	0.058	0.090	0.080	0.042	0.080	0.058	0.075	0.058	0.080	0.019
3.10	0.058	-	-	-	0.042	0.048	0.038	-	-	0.042	-	0.014
3.11	0.078	0.088	0.088	0.094	0.094	0.094	0.086	0.091	0.086	0.081	0.097	0.005
3.13	0.083	-	-	0.075	0.083	0.091	0.079	-	-	0.074	-	0.008
ii *"Residual" Zn*												
3.9	0.058	0.037	0.042	0.026	0.042	0.032	0.048	0.069	0.048	0.037	0.058	0.013
3.12	0.083	0.073	0.073	0.062	0.091	0.087	0.071	0.085	0.070	0.081	0.081	0.008

Appendix 4.1 Relationship between Cd concentration in grain and soil pH_{Ca} for (a) the 1986 and (b) 1987 harvests of Experiment 4.1 and (c) the 1990 harvest of Experiment 4.2 for each rotation

(a) 1986 harvest of Experiment 4.1

Crop Rotation	Slope ± SD	Intercept	R^2 (adjusted)	Significance
wheat-wheat	-0.02 ± 0.08	0.016	0.03	-
wheat-barley	0.08 ± 0.03	-0.005	0.02	-
wheat-peas	0.06 ± 0.06	0.002	0	-
wheat-lupins	0.04 ± 0.03	0.012	0.19	-
wheat-beans	0.03 ± 0.04	0.006	0	-
wheat-volunteer pasture	0.04 ± 0.04	0.003	0	-
wheat-sown pasture	0.07 ± 0.05	-0.002	0	-
wheat-fallow	0.11 ± 0.03	-0.010	0.23	-

(b) 1987 harvest of Experiment 4.1

wheat-wheat	0.11 ± 0.05	-0.008	0	-
wheat-barley	0.08 ± 0.03	-0.005	0	-
wheat-peas	0.04 ± 0.07	0.007	0	-
wheat-lupins	0.41 ± 0.07	-0.062	0.63	***
wheat-beans	0.11 ± 0.03	-0.009	0.15	-
wheat-volunteer pasture	0.03 ± 0.04	+0.004	0	-
wheat-sown pasture	0.09 ± 0.02	-0.004	0	-
wheat-fallow	0.09 ± 0.03	-0.007	0.09	-

(c) 1990 harvest of Experiment 4.2

wheat-wheat	0.10 ± 0.07	-0.015	0.01	-
wheat-pasture	-0.04 ± 0.03	+0.014	0.31	*
wheat-lupins	0.04 ± 0.05	-0.001	0	-

Appendix 5.1 Determination of the quantities of sulphur and calcium carbonate required to adjust soil pH by a designated amount for each soil used in the glasshouse experiment.

5.2.1 Introduction

The four soils used in the glasshouse experiment had different buffering capacities. Thus it was necessary to determine buffer curves for each soil in order to calculate the quantities of sulphur(S) and calcium carbonate ($CaCO_3$) required to adjust the pH to a designated value.

5.2.2 Materials and Methods

Since only limited time was available to determine the buffer curves it was necessary to hasten the reactions of $CaCO_3$ with the soil. The rapid method for determining titration curves by Barrow & Cox (1990) was used. This involved incubating samples of the four soils (50g) with varying amounts of $CaCO_3$ to give the following range (mmol $(^+)kg^{-1}$) : 0, 10, 25, 40, 50, 100 and 150. Approximately five grams of soil in triplicate was removed at the following time intervals (5, 18 and 36 hours) to determine the buffer curves.

5.2.3 Results and Discussion

Titration curves for the four soils are shown in Figs 5.1.1 - 5.1.4. From the titration curves the quantity of $CaCO_3$ (mmol $(^+)$/kg) required to raise the soil pH by one unit was determined as follows.

Table 5.2.1 **Quantities of $CaCO_3$ required to raise the soil pH by one unit for each of the 4 soils used in the glasshouse study.**

Soil	$CaCO_3$ mmol$(^+)kg^{-1}$
Inman Valley	56
Kapinnie	14
Freeling	14
Bordertown	19

Examples of calculations to determine the quantities of $CaC0_3$ and S to add are given below.

$CaC0_3$ Additions

Freeling requires 14 mmol $(^+)$ $CaC0_3$ kg^{-1} soil to raise the soil pH one unit (from Table 5.2.1).

Therefore to raise the pH from 4.75 to 6.0 i.e. 1.25 units the following quantity of $CaC0_3$ is required:

$$1.25 \text{ x } 14 \text{ mmol } (^+) \ CaCO_3 \ kg^{-1}$$

$$= 17.5 \text{ mmol } (^+) \ CaC0_3 \ kg^{-1}$$

$$\frac{17.5\text{mmol kg}^{-1}}{1000 \text{ mmol mol}^{-1}} \quad \text{x} \quad \frac{100 \text{ (molecular weight } CaC0_3)}{2 \text{ (divalent cation)}}$$

Sulphur Additions

Addition of $CaCO_3$ results in the following reaction:

$$CaCO_3 + H_2O \leftrightarrow Ca^{2+} + HCO_3^- + OH^-$$

$$2H^+ + HCO_3^- + OH^- \leftrightarrow 2H_2O + CO_2$$

Thus one mole of $CaCO_3$ produces one mole OH^- and consumes two moles H^+.

Addition of S results in the following reaction

$$H_2 + S + 2O_2 \leftrightarrow H_2SO_4$$

$$H_2SO_4 \leftrightarrow 2H^+ + SO_4^{2-}$$

Thus one mole of S produces two moles H^+.

Thus sulphur equivalent for calcium carbonate equivalents are in a 1:1 ratio i.e. 100g $CaCO_3$ is equivalent to 32g S for the purposes of calculating the quantity of S required to adjust soil pH.

Freeling requires 14 mmol (+) $CaCO_3$ kg^{-1} soil to raise the pH one unit. Therefore to lower pH from 4.75 to 4.5 i.e. 0.25 pH units the following quantity of S is required:

$$0.25 \times 14 \text{ mmol } (+) \; CaCO_3 \; kg^{-1}$$

$$= 3.5 \text{ mmol } (+) \; CaCO_3 \; kg^{-1}$$

$$= \frac{3.5}{1000} \times \frac{98}{2}$$

$$= 0.175 \text{ g } CaCO_3 \; kg^{-1}$$

Therefore the quantity of S required $= 0.175\text{g} \times \dfrac{32 \text{ (atomic weights)}}{100 \text{ (molecular weight } CaCO_3)}$

$= 0.0571\text{gS } kg^{-1}$.

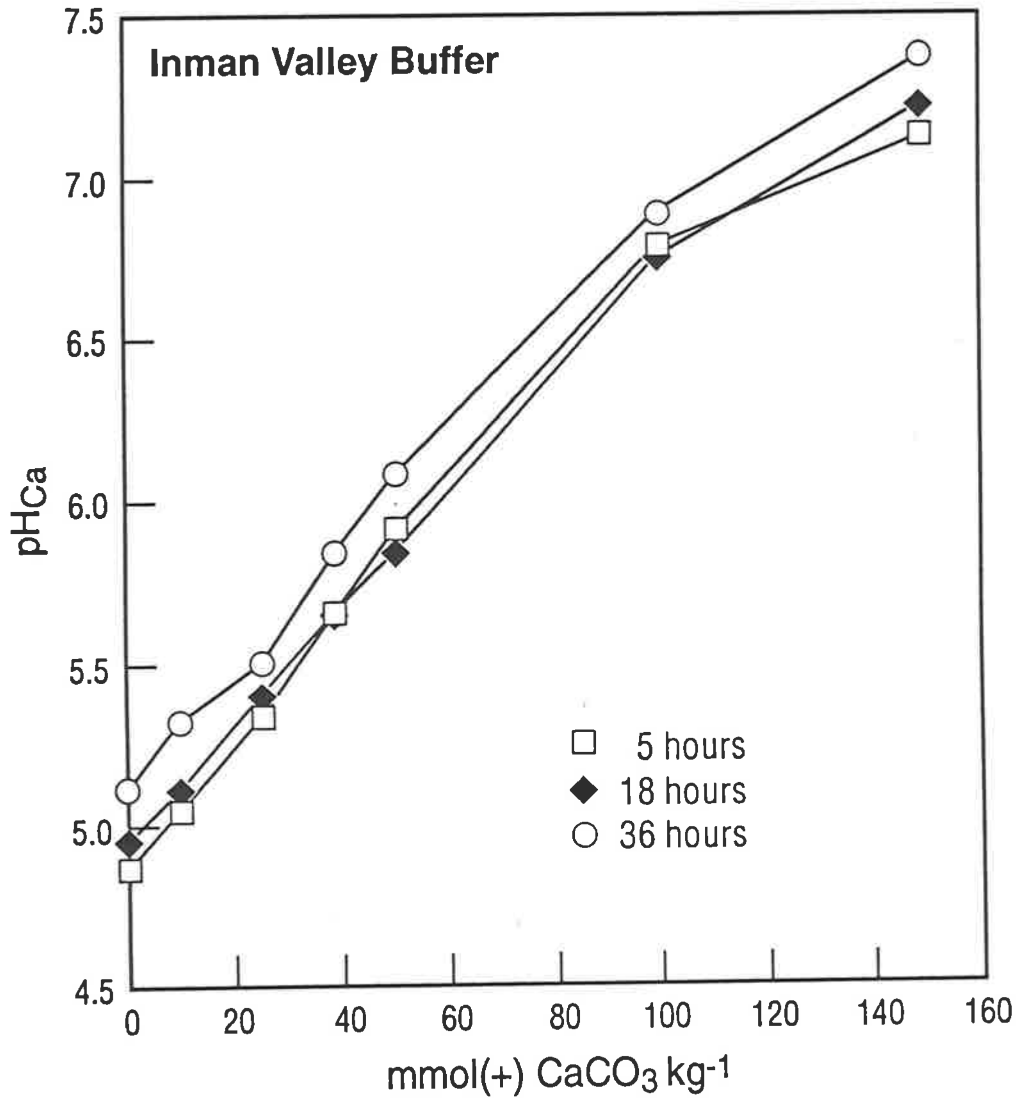


Figure 5.1.1. Titration curve for the Inman Valley soil.

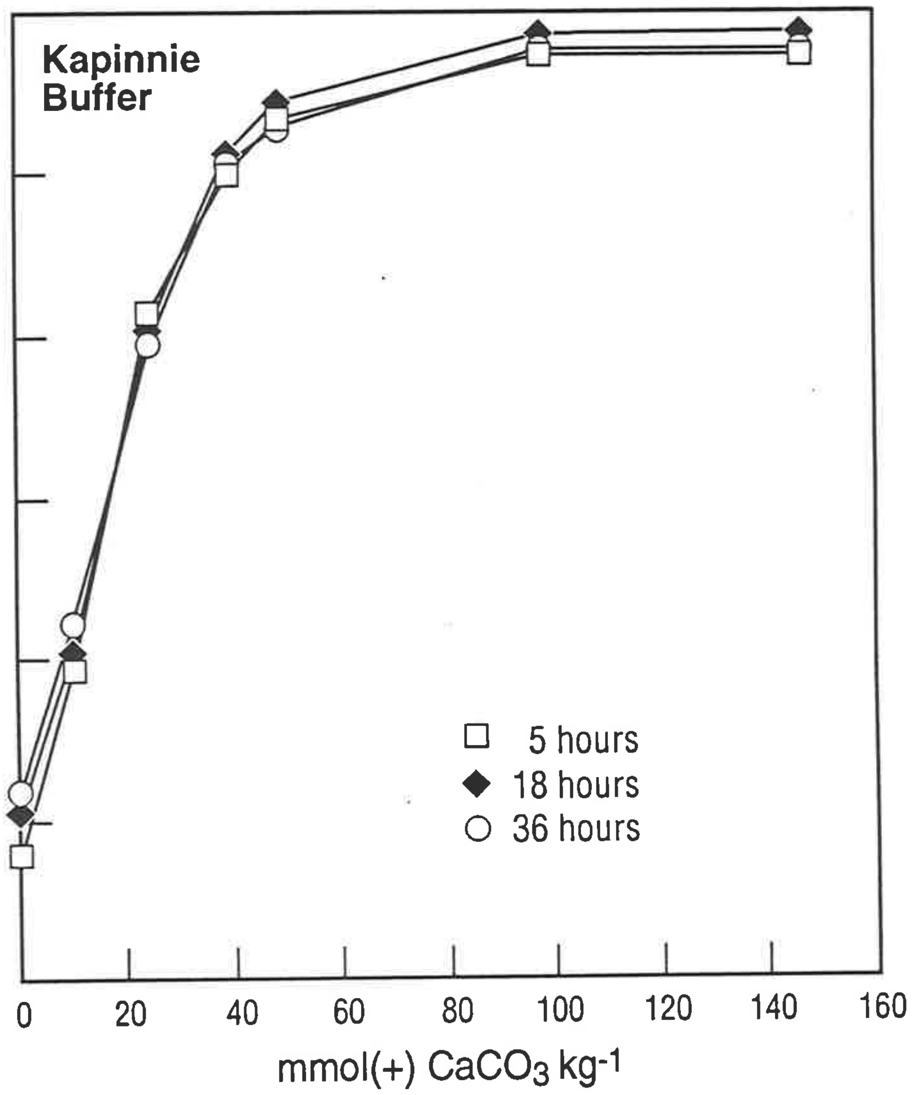


Figure 5.1.2. Titration curve for the Kapinnie soil.

D.Oliver, figure 5.1.1 & 5.1.2. D99/GER.

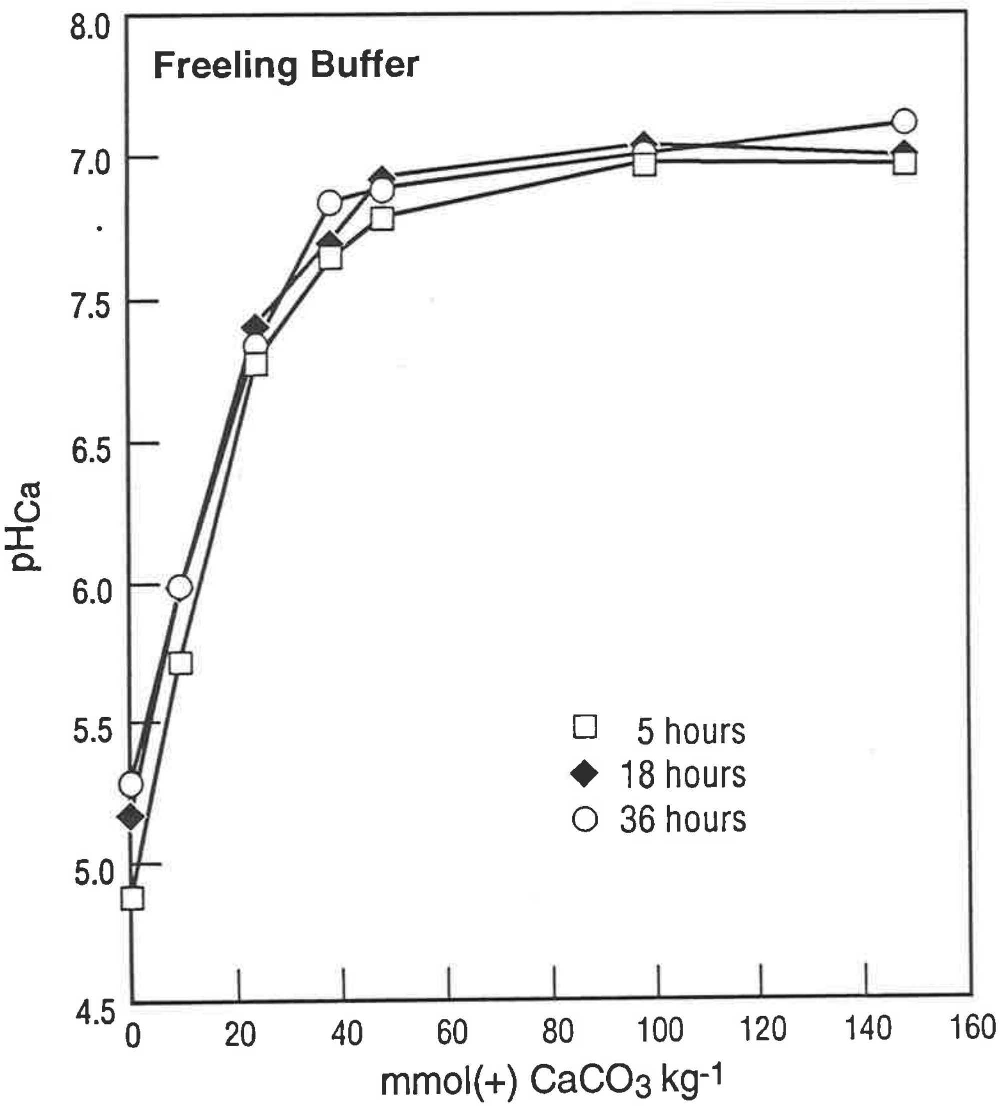


Figure 5.1.3. Titration curve for the Freeling soil.

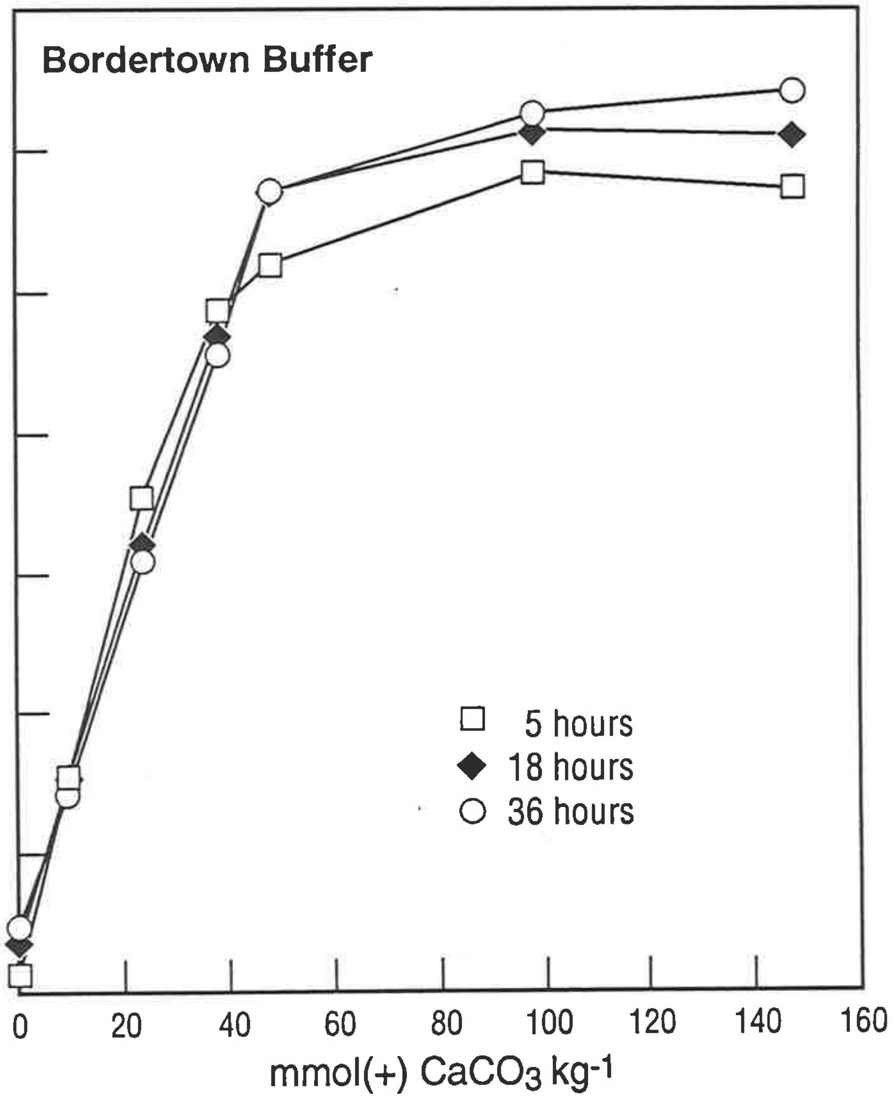


Figure 5.1.4. Titration curve for the Bordertown soil.

D.Oliver, figure 5.1.3 & 5.1.4. D99/GER.

Appendix 5.2 Preparation of Cd treatments in the glasshouse experiment

5.2.1 Introduction

An inert carrier medium (sand) was used to add the cadmium treatments to each pot to ensure the cadmium treatments were thoroughly mixed throughout the soil.

5.2.2 Material and Methods

Preliminary Treatment of Sand

Several kg of sand were sieved through a 2 mm stainless steel mesh to remove any "large" debris. The sand was then heated to 1050-1200°C in a muffle furnace to ignite any organic matter and then acid washed in 2M HCl to desorb any metals bound to the sand surface. The acid was then removed from the sand by washing the sand many times with deionised water. The removal of the acid was determined by measuring the pH of the deionised water when mixed through the sand and noting when the water pH had returned to neutral. The sand was then dried at 40°C for several days and ground finely in a ceramic mixer with ceramic balls.

Application of Cd stock solution

A stock solution of cadmium was made by dissolving 10.973 g $CdCl_2.2^1/_2H_2O$ in 200 mL dilute nitric acid. The following quantities of stock solution were dripped onto 1.0 kg sand to give the bulk sand samples with varying cadmium treatments. Varying sub-samples of the bulk sand samples were then added to each pot for a specified cadmium treatment depending upon the mass of soil in each pot (Appendix 5.3).

Table 5.3.1 Volume of stock solution (mL) added to 1.0 kg acid-washed sand for each bulk sample of cadmium-treated sand.

Quantity bulk sand	**Volume of stock solution**	**Mass $CdCl_2$ $2^1/_2H_2O$ in 1000 g sand**	**Quantity Cd (mg) per kg soil**
kg	mL	g	mg kg^{-1}
1.0	50.00	2.743	2.7
1.0	16.67	0.914	0.9
	STOCK SOLUTION DILUTED $^1/_5$		
1.0	27.78	0.305	0.3
1.0	9.26	0.102	0.1

The nil Cd treatment was made by dripping 30 mL of 5% V/V HNO_3 onto 1.0 kg of sand.

After the Cd was added the sand was dried at 40°C in an oven and then re-ground and mixed thoroughly using the ceramic balls and mixer. The bulk sand samples (3 g) were digested with concentrated nitric acid (7 mL) and analysed by graphite furnace or flame atomic absorption to determine the actual quantities of cadmium in the sand. Results from the digestion analysis were:

Table 5.3.2 **Summary of the Cd concentration added to each soil in the glasshouse experiment.**

Treatment Applied to Sand	Cadmium concentration	Cadmium concentration when 10g sand mixed through 5.0kg soil
	μg Cd g^{-1} sand	*μg Cd kg^{-1} soil*
none (sand only)	0.0125	0.0250
dilute acid (ie nil Cd treatment)	0.0175	0.0350
		mg Cdkg^{-1} soil
0.1 mg kg^{-1} Cd	50.0	0.10
0.3 mg kg^{-1} Cd	150.0	0.30
0.9 mg kg^{-1} Cd	458	0.92
2.7 mg kg^{-1} Cd	1433	2.87

Appendix 5.3 Quantities of sand (mg pot^{-1}) to which a stock quantity of $CdCl_2.2H_2O$ had been added and that were mixed through the soil in each pot to give the designated Cd treatment (mg kg^{-1}).

Cd Treatments (mg kg^{-1})

SOIL	**Mass soil in pot (kg)**	0	0.1	0.3	0.9	2.7
				g sand pot^{-1}		
Inman Valley	3.75	7.5	-	7.5	7.5	7.5
Kapinnie	4.75	9.5	9.5	9.5	9.5	-
Freeling	5.0	10.0	-	10.0	10.0	10.0
Bordertown	5.0	10.0	10.0	10.0	10.0	-

Appendix 5.4 Mean number of tillers produced per pot by plants grown in each sulphur (S) or calcium carbonate (CaCO$_3$) treatment.

	Inman Valley	Kapinnie	Freeling	Bordertown
	0.96 g S kg^{-1} 21.19	0.34 g S kg^{-1} 16.94	0.26 g S kg^{-1} 13.00	0.23 g S kg^{-1} 10.06
	Nil 12.50	0.17 g S kg^{-1} 14.69	0.09 g S kg^{-1} 11.81	Nil 9.56
	0.84 g CaCO$_3$ kg^{-1} 15.50	Nil 13.69	Nil 12.44	0.48 g CaCO$_3$ kg^{-1} 9.25
	2.24 g CaCO$_3$ kg^{-1} 14.44	0.35 g CaCO$_3$ kg^{-1} 13.13	0.53 g CaCO$_3$ kg^{-1} 12.25	0.95g CaCO$_3$ kg^{-1} 9.56
	3.64 g CaCO$_3$ kg^{-1} 13.69	0.70 g CaCO$_3$ kg^{-1} 13.69	0.88 g CaCO$_3$ kg^{-1} 12.44	1.43 g CaCO$_3$ kg^{-1} 11.25
s.e.d.	0.77	0.51	0.44	0.44

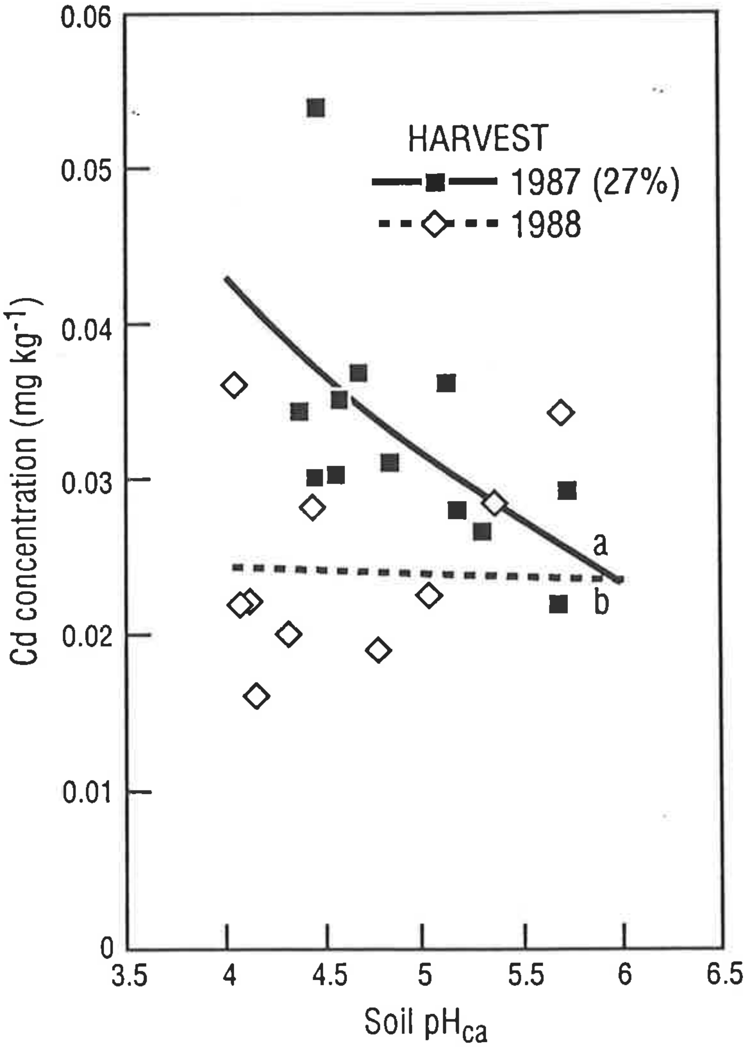
Appendix 5.5 Mean grain number produced for each sulphur (S) or calcium carbonate (CaC0$_3$) treatment for each soil (g kg^{-1} soil).

	Inman Valley	Kapinnie	Freeling	Bordertown
	0.96 g S kg^{-1} 641.06	0.34 g S kg^{-1} 442.81	0.26 g S kg^{-1} 425.63	0.23 g S kg^{-1} 381.88
	Nil 557.44	0.17 g S kg^{-1} 417.63	0.09 g S kg^{-1} 425.31	Nil 388.06
	0.84 g CaCO$_3$ kg^{-1} 597.63	Nil 464.44	Nil 445.25	0.48 g CaCO$_3$ kg^{-1} 372.50
	2.24 g CaCO$_3$ kg^{-1} 601.69	0.35 g CaCO$_3$ kg^{-1} 452.00	0.53 g CaCO$_3$ kg^{-1} 418.63	0.95g CaCO$_3$ kg^{-1} 373.75
	3.64 g CaCO$_3$ kg^{-1} 575.50	0.70 g CaCO$_3$ kg^{-1} 493.94	0.88 g CaCO$_3$ kg^{-1} 433.44	1.43 g CaCO$_3$ kg^{-1} 432.38
s.e.d.	27.09	16.84	15.70	11.67

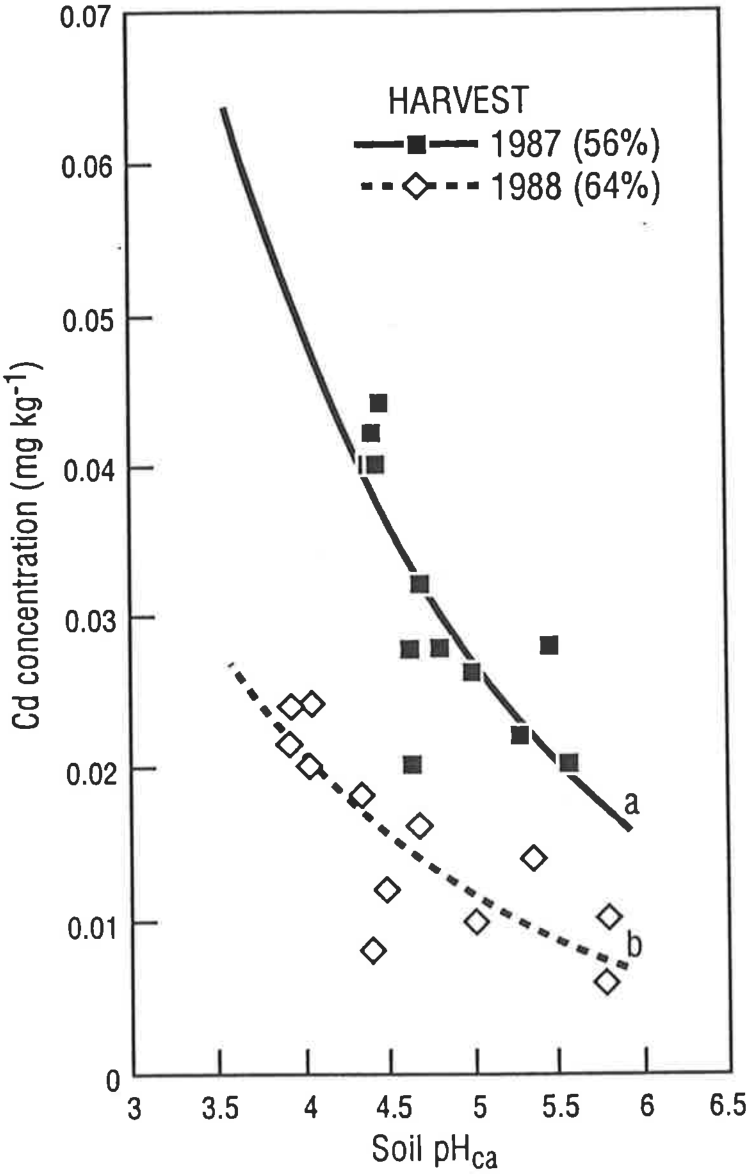
Appendix 5.6 Exponential equations for the relationship between Cd concentration in grain and soil pH for plants grown in the Inman Valley and Kapinnie soil.

The exponential equation is:
grain Cd concentration = $a + b \times \exp\,(^{-k})^{\,\mathrm{pH}}$

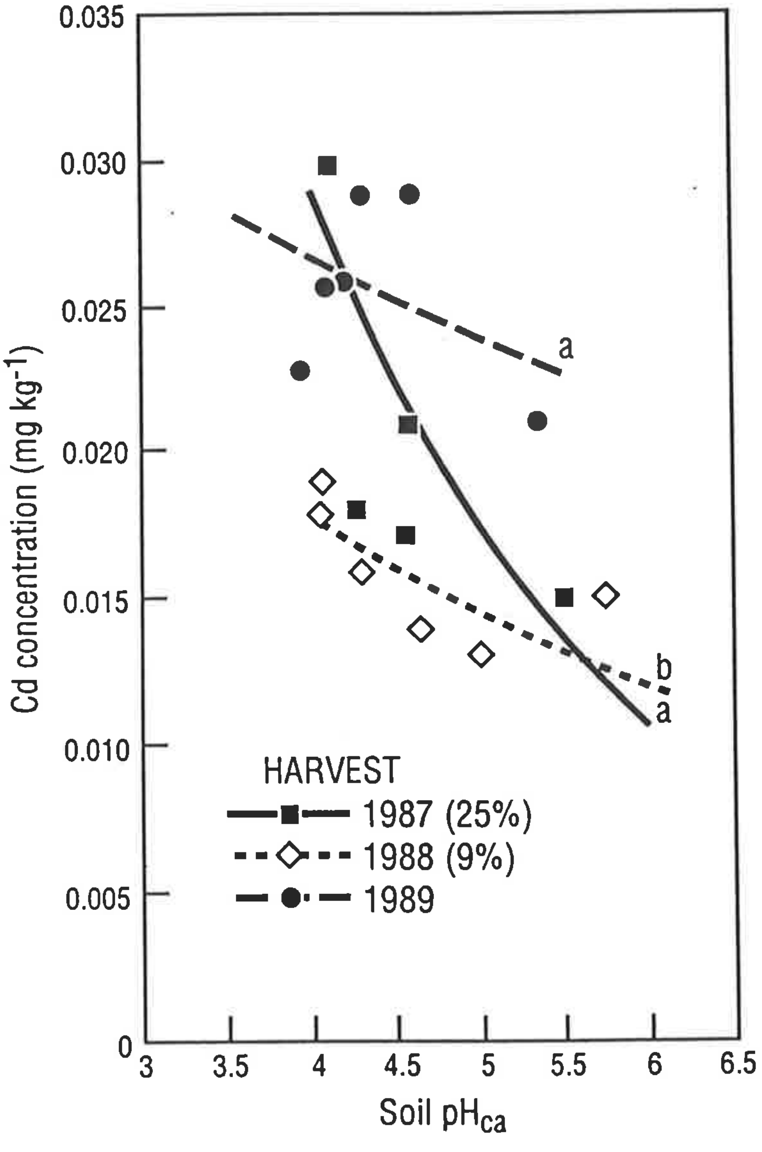
Soil	Cd treatment	a	b	exp^{-k}	percent variance account for by model
Inman Valley	2.7	1.4286	-2.27 E +06	0.0236	53.1
Kapinnie	0.9	0.6695	3.84 E +09	0.0046	90.2



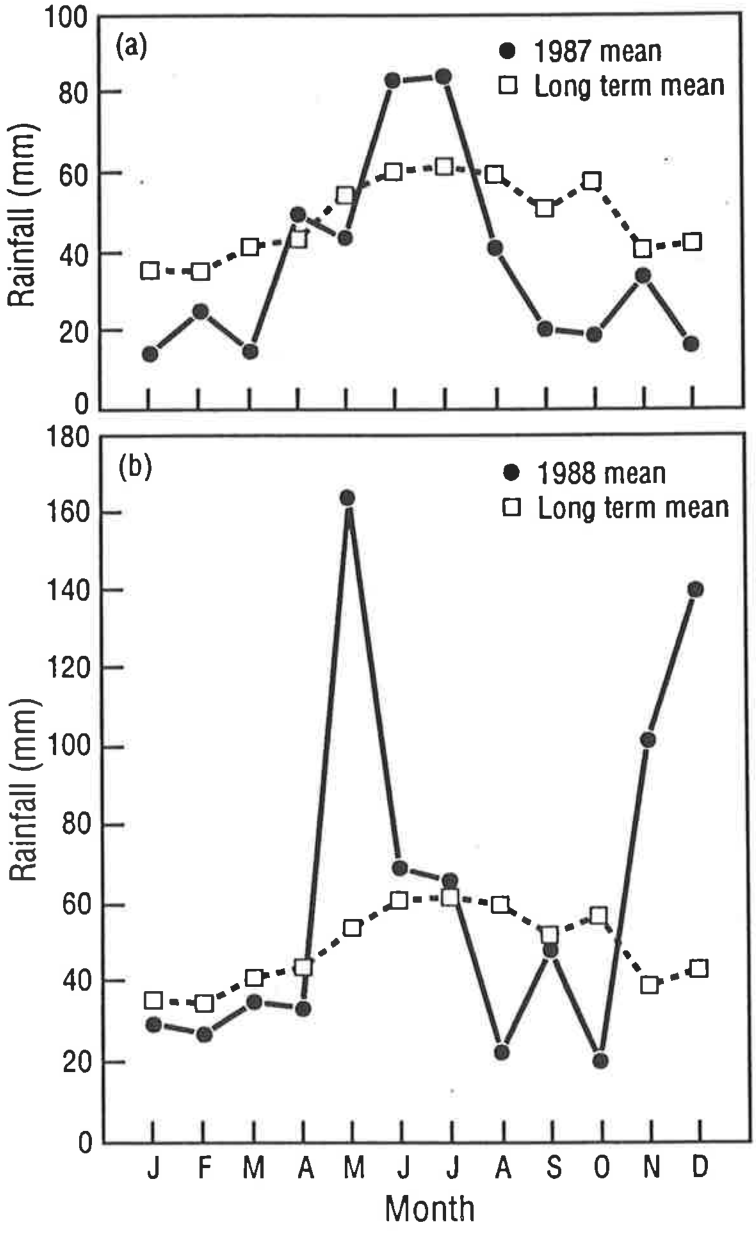
Appendix 6.1. Variation in grain Cd concentration (mg kg^{-1}) with pH for Experiment 6.1 (Devenish, Vic.). All data was fitted to an exponential model and are presented with the concentrations. In Appendices 6.1-6.3 statistical differences between years are distinguished by letters and the percent variance accounted for by the exponential model is given in brackets. When the data did not fit the model, no value is given.



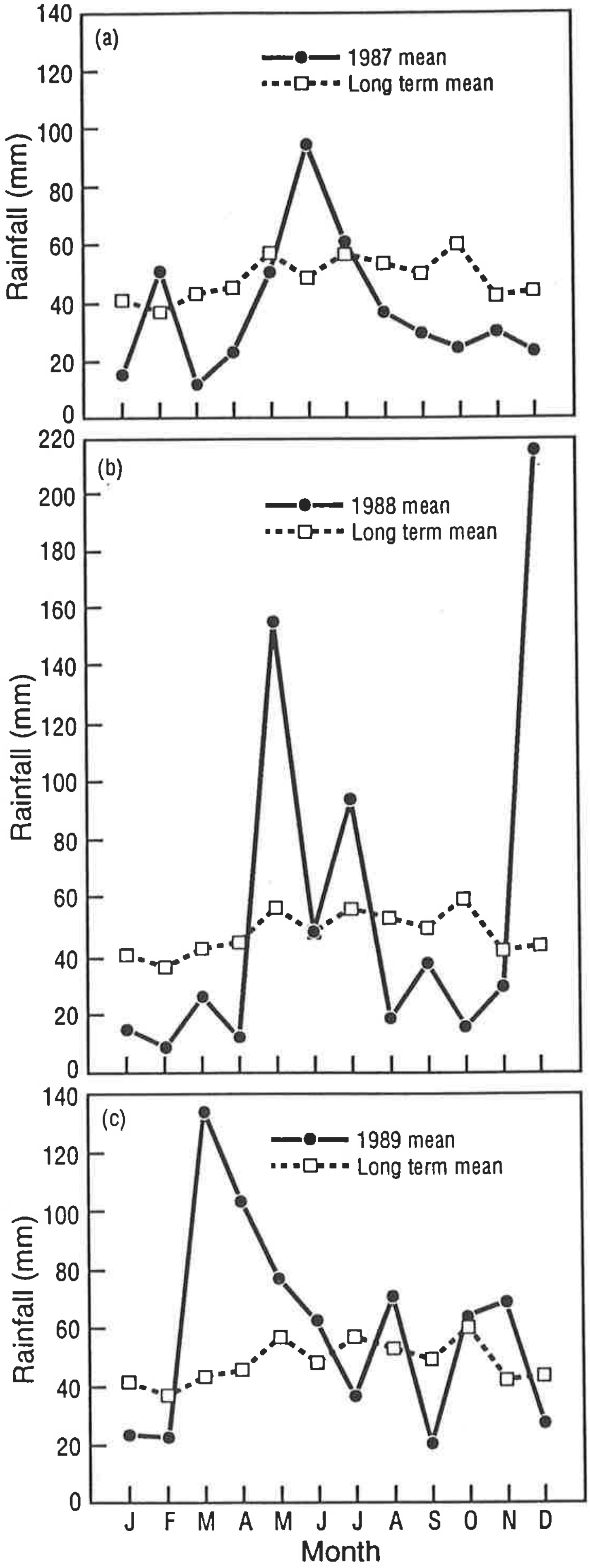
Appendix 6.2. Variation in grain Cd concentration (mg kg^{-1}) with pH for Experiment 6.3 (Burramine, Vic.). All data was fitted to an exponential model and are presented with the concentrations.



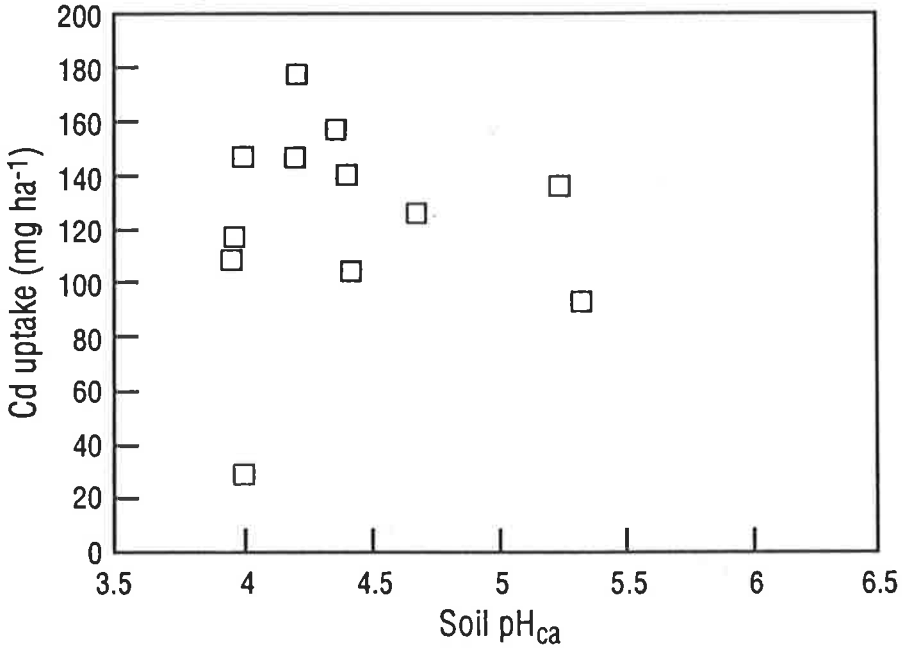
Appendix 6.3. Variation in grain Cd concentration (mg kg^{-1}) with pH for Experiment 6.8 (Book Book, NSW). All data was fitted to an exponential model and are presented with the mean concentrations.



Appendix 6.4. Monthly average rainfall for (a) 1987 and (b) 1988 and long-term monthly average rainfall for Rutherglen.



Appendix 6.5. Monthly average rainfall for (a) 1987, (b) 1988 and (c) 1989 and long-term monthly average for Wagga Wagga.



Appendix 6.6. Variations in Cd uptake (mg ha^{-1}) with pH for the 1989 harvest of Experiment 6.7.

Appendix 7.1 Published Manuscripts and Conference Proceedings from this Study.

Refereed Manuscripts

1. **Oliver, D.P.,** Schultz, J.E., Tiller, K.G. and Merry, R.H. (1993). The effect of crop rotations and tillage practices on cadmium concentrations in wheat grain. *Aust. J. Agric. Res.* **44**, 1221-34.

2. **Oliver, D.P.,** Hannam, R., Tiller, K.G., Wilhelm, N.S., Merry, R.H. and Cozens, G.D. (1994). The effects of zinc fertilization on cadmium concentration in wheat grain. *J. Environ. Qual.* (in press)

Refereed Conference Proceedings

1. **Oliver, D.P.,** Tiller, K.G., Conyers, M.K. Slattery, W.J., Merry, R.H. and Alston, A.M. (1993). The effects of soil pH on cadmium concentration in wheat grain grown in the field. Third Int. Symp. on Plant-Soil Interactions at Low pH. (in press).

2. **Oliver, D.P.** and Tiller, K.G. (1993). Management factors that affect cadmium uptake by wheat grain. Proc. of 7th Australian Agron. Conf., University of Adelaide, South Australia. September 19-24, 1993. pp. 122-5.

3. Tiller, K.G., **Oliver, D.P.**, McLaughlin, M.J., Merry, R.H. and Naidu, R. (1993). Managing cadmium contamination of agricultural land. Proc. of 2nd Int. Conf. on the Biogeochem. of Trace Elements. Taiwan, Sept. 1993. (in press)

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Basta, N.T., and Tabatabai, M.A. (1992b). Effect of cropping systems on adsorption of metals by soils. II. Effect of pH. *Soil Sci.* **153**, 195-204.

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