Zinc Application and Its Availability

to Plants

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I declare that this thesis is my own work and contains as its main content, work which has not been submitted for a degree at any other tertiary institution.

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Summary of thesis

Globally, low zinc (Zn) soils are widespread, but one of the largest expanses of such soils is in south west Australia (WA). Early Zn research in the region determined how much fertiliser Zn was required for profitable production of spring wheat (*Triticum aestivum* L.) and subterranean clover (*Trifolium subterraneum* L.), the major crop and pasture species at the time. The research showed that Zn sulfate and ZnO were equally effective Zn fertilisers, but ZnO was cheaper and so was widely used. The research indicated that in the year of application, depending on soil type, between 0.5-1.5 kg Zn/ha provided adequate Zn for the production of wheat and subterranean clover. The length of time that a single application of Zn fertiliser remains fully effective in maintaining the production of crops and pasture in future years (residual value; (RV)) had not been determined. This knowledge of the RV of Zn fertilisers is required for soils of WA. The experiments that measured the RV of fertiliser Zn for spring wheat and subterranean clover form the bulk of this thesis.

The soils in the region were also initially acutely phosphorus (P) deficient requiring the application of fertiliser P for profitable production. Single superphosphate was the P fertiliser initially used. It was manufactured locally using phosphate rock imported from Nauru and Christmas Islands. This phosphate rock also contained much Zn, and the single superphosphate manufactured from it contained 400-600 mg Zn/kg. At amounts of application needed to provide adequate P, the Zn-contaminated superphosphate also supplied about 90 g Zn/ha. Therefore, early field experiments measured the RV
of ZnO applied to soil when single superphosphate was applied annually at 
>150 kg/ha. In these experiments, the RV of Zn was measured when different 
amounts of fertiliser nitrogen (N) was applied. This was because it has recently 
been very profitable to apply fertiliser N to wheat crops, which greatly 
increased grain yields and so may have increased the demand for Zn, thereby 
probably decreasing the RV of the original ZnO application. In these 
experiments, there were many nil-Zn plots. In subsequent years, freshly-
applied ZnO amounts were applied to measure the RV of the original ZnO 
treatments relative to the fresh Zn treatment. No Zn deficiency was detected for 
up to 23 years after applying ZnO while applying superphosphate at >150 kg/ha 
per year and for all amounts of N applied.

Subsequently cheap imported DAP fertiliser was used for wheat crops instead 
of locally produced Zn-contaminated single superphosphate and urea. The 
imported DAP contained about 50 mg Zn/kg (1/12 that of single 
superphosphate). This new fertiliser strategy induced Zn deficiency in many 
wheat crops. This led to further field studies to determine the RV of ZnO 
fertiliser when DAP was applied. The experiments also included 2 Zn-
contaminated single superphosphate treatments. In one, no ZnO was applied, 
and superphosphate was applied at >150 kg/ha per year to match the amount of 
P applied as DAP to the other treatments. The other treatment was the same, 
except 1.5 kg/ha Zn as ZnO was applied in the first year only. In subsequent 
years, freshly-applied ZnO amounts were applied to measure the RV of the 
original ZnO treatments relative to the fresh Zn treatment. Relative to freshly-
applied Zn in each year, the RV of the original ZnO treatments decreased as the length of time that the Zn was in contact with soil increased. However, the rate of decline in the RV was also found to differ with soil type, and was affected by soil pH, clay and organic carbon content of soil, and in alkaline soils with the calcium carbonate content of soil.

Parallel glasshouse studies measured the RV of Zn, as Zn sulfate, for wheat and subterranean clover, using many soils from WA and other Australian States. The glasshouse studies also showed that the rate of decline in the RV of the original Zn application varied markedly with soil type and was strongly influenced by soil pH, clay and organic carbon content of soil, and in the alkaline soils, the amount of calcium carbonate in soil.

In the above studies, the RV of fertiliser Zn was measured relative to freshly-applied Zn using yield of plants (shoots and grain for wheat, shoots for clover), Zn content in shoots and grain, and soil test Zn using the ammonium oxalate and DTPA procedures. In addition, Zn concentration in young tissue and rest of shoots (glasshouse studies) and young tissue and whole shoots (field studies) was measured, and Zn concentration related to 90 % of the maximum yield (critical Zn in plant parts) was determined. The studies showed that the DTPA soil test procedure, together with soil pH, and clay and organic matter content of soil, was an accurate prognostic test for indicating when Zn deficiency was likely in the next clover or wheat crop. The study confirmed that young tissue (youngest fully expanded leaves) provided critical plant test values for
diagnosing Zn deficiency in plants. The plant and soil tests for Zn are now used by commercial soil and tissue testing laboratories.

When Zn deficiency was diagnosed early in field grown wheat, Zn sprays can be applied to the crop foliage to prevent or minimise decreases in grain yields at the end of the growing season. Zn sulfate and Zn chelate are the most widely used compounds. This thesis reports the results of a field study to compare the effectiveness of the two compounds when the spray was applied at two growth stages of wheat (Gs14; seedling growth and Gs24; tillering). In addition, Zn applied with the seed while sowing the wheat crop was also included. Zinc applied to the soil while sowing was the most effective treatment. Zn chelate was more effective as a spray than Zn sulfate when applied at the earlier growth stage, but Zn sulfate was cheaper, and both sprays were equally effective when applied at the later growth stage.

Recently in the region, durum wheat (*T. durum* L.), narrow-leafed lupin (*Lupinus angustifolius* L.), yellow lupin (*L. luteus* L.), white lupin (*L. albus* L.), canola (*Brassica napus* L.), chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.) and lentil (*Lens culinaris* Medik) were all increasingly grown in rotation with spring wheat. Consequently, the Zn requirement of the new crops was compared with the Zn requirements of spring wheat. Species requiring less Zn than spring wheat to produce the same relative yield were faba bean, chickpea, albus lupin and canola; species requiring more Zn were lentil and durum wheat.
Spreadsheet models were developed to determine when re-application of fertiliser Zn was required for low and high production systems. Relative to freshly-applied Zn, the rate of decline in the RV of Zn applied in a previous year varied depending on the amount of Zn applied, time the Zn was in contact with soil since application, properties of the soil (soil pH, % clay, % organic carbon, % free calcium carbonate), plant species, and the amount of Zn removed in harvested grain or hay.

The thesis has culminated in a better understanding of Zn in the agricultural production systems of WA. The distribution and correction of Zn deficiency is now predictable for the many soil types and cropping systems of WA. Accurate identification of Zn deficiency for a range of crop and pasture species by plant analyses, typically the youngest mature leaf, is now possible for local conditions. With the calibration of the DTPA Zn soil test for soils of WA, particularly for wheat the major crop species grown in WA, prognosis of potential Zn deficiency can now be predicted before the appearance of Zn deficiency or loss in plant production.
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Publications

The publications listed below form the basis for the major part of this thesis.


Brennan RF, Bolland MDA (2002) Relative effectiveness of soil applied zinc
Chapter 1

Literature Review

1.1 Essentiality of Zn

Zinc (Zn) is an essential micronutrient (trace element or minor element) for humans, animals and plants (Sommer 1928; Sauchelli 1969; Lisk 1971; Knezek and Ellis 1980; Nriagu 1980; Miller et al. 1991; Fageria et al. 2002). While the beneficial effect of Zn on the growth of Aspergillus niger was known in about 1870, the essentiality of Zn for higher plants is generally attributed to Sommer and Lipman (1926). In Australia, the first recorded increase in plant growth to Zn fertiliser was for citrus (Pitman and Owen 1936). More detailed historical information on the importance of Zn for plants is available elsewhere (Viets 1966; Bauer 1971).

Higher plants generally absorb Zn as a divalent cation (Zn$^{2+}$) which acts either as a metal component of enzymes or as a functional, structural, or a regulatory cofactor of a large number of enzymes (Kessler and Monselise 1959; Kessler 1961; Price et al. 1972; Nicholas 1975; Vallee and Wracker 1976; Vallee 1983; Brown et al. 1993). In grasses, it has been suggested that non-protein amino acids, called phytosiderophores can complex Zn$^{2+}$ in the rhizosphere and transport Zn into the root cell (Kochian 1993). At least four enzymes contain bound Zn: carbonic anhydrase (Wood and Sibley 1950; Atkins et al. 1972; McMill and Bouma 1973; Marschner 1986), alcohol dehydrogenase (Marschner 1986), Cu-Zn superoxide dismutase (Marschner 1986; Cakmak and Marschner 1987; Cakmak et al. 1989) and RNA polymerase (Soloiman and Wu 1985; Marschner 1986; Obata and Umebayashi 1988). Furthermore, Zn is required for the activity of various enzymes, such as dehydrogenases, aldolases, isomerases, transphosphorylases, RNA and DNA polymerases (Kessler and Monselise 1959; Kessler 1961). Zinc is involved in
carbohydrate and protein metabolism (Jyung et al. 1975). Zinc is also required for the synthesis of tryptophan, a precursor for the synthesis of indoleacetic acid (Cakmak et al. 1989). Zinc appears to have an active role in the production of auxins for a range of plant species (Skoog 1940; Tsui 1948a). The most pronounced Zn deficiency symptom of stunted growth and ‘little leaf rosette’ of plants, particularly fruit trees (Chandler et al. 1931), appears to be related to the physiological function of Zn in auxin production (Spiller and Terry 1974). Giberellic acid metabolism seems impaired by Zn deficiency in plants (Skoog 1940; Suge et al. 1986). Tsui (1948b) first suggested the role of Zn in the water relations and osmotic pressure of tomato plants.

Zinc appears to be involved in cell membrane proteins probably due to Zn preferential binding with SH- groups, and there is loss of membrane integrity in Zn–deficient plants (Chvapil 1973; Cakmak and Marschner 1988a, b, 1990). Zinc appears to have a special role in regulating the uptake of nutrients as Zn treatments produced an increase of P and Mn concentrations in barley shoots before differences in dry weight of shoots were measured (Norvell and Welch 1993; Welch and Norvell 1993).

A relationship was established between carbonic anhydrase, a Zn requiring enzyme, and the regulation of photosynthesis, suggesting Zn involvement in the photosynthesis reaction of the plant (Randall and Bouma 1973; Graham and Reed 1991). Sharma et al. (1995) indicated the involvement of Zn in stomatal opening, possibly as a constituent of carbonic anhydrase needed to maintain adequate HCO₃⁻ in the guard cell, and also Zn affected the influx of K⁺ uptake into guard cells.

Welch and Norvell (1993) suggested that Zn had a protective role in preventing the oxidation of sulfhydryl groups to disulfides in the root cell membrane. Recently, Zn has been suggested to play roles in protecting cells by both controlling generation as well as detoxification of reactive oxygen species (Cakmak and Marschner 1988c; Cakmak 2000).
1.2. Geographic Distribution of Zn deficient soils

Zinc deficiency in plants is widespread throughout the world (Viets 1966; Lindsay 1972a; Bould et al. 1983; Welch et al. 1991; Takkar and Walker 1993). Usually, Zn deficiency in plants is associated with calcareous high pH soils because of low Zn availability or with coarse textured (sandy), highly leached, acid soils because of their low total Zn content (Takkar and Walker 1993). Negative relationships between Zn and several other essential elements (e.g. P, N, Cu) can also lead to Zn deficiencies in plants (Hewitt 1963; Robson and Pitman 1983; Soliman and Farrah 1987; Loneragan and Webb 1993).

Sillanpää (1982) reported that Zn deficiency in plants was recorded in almost every country studied, excluding Belgium and Malta. The lowest soil Zn levels were found in Iraq, Turkey, India, Pakistan, Syria, Lebanon, Mexico, Italy, Nepal, Tanzania, and Thailand (Sillanpää 1972, 1982). The most prominent areas of Zn deficient soils in the tropics occurred in Brazil (Sanchez and Cochrane 1980; Sillanpää and Vlek 1985), Africa (eg Chad, Nigeria, Sth. Africa) (Ferrand et al. 1951; Stanton and Burger 1966; Egbe and Omotosa 1972; Udo and Fagbami 1979; Cottenie et al. 1981; Kayode and Agboola 1983; Adetunji 1999), India (Rathore et al. 1978, 1980), and the Philippines (Katyal and Vlek 1985). Extensive areas of the calcareous soils of Turkey are Zn deficient for a range of crops (Cakmak et al. 1999). However, the Zn status of the Llanos region of the Amazon basin (Latin America), the Congo basin (Africa), and the Indo-Chinese Peninsula (Asia) appears not to have been studied systematically (Welch et al. 1991). In Africa, Zn deficiencies have been reported in Nigeria, Guinea, the Ivory Coast, Sierra Leone, Sudan, and Zimbabwe (Kang and Osiname 1985). In many of these countries, Zn deficiency has also been induced by the use of lime to increase the soil pH value (pH effects on Zn availability see section 1.6.5).
1.2.1 Distribution of Zn deficient soils in USA

The geographic pattern of Zn deficiency in plants grown in soils of the USA is poorly defined (SSSA 1965; Hodgson et al. 1971; Kubota and Allaway 1972; Karim and Sedberry 1976; Welch et al. 1991). This is largely due to the frequency of small local Zn-deficient areas in various regions of the USA. The geographic pattern of the distribution of Zn deficient soils can be influenced by various environmental factors that reduce or enhance Zn availability to plants, or by differences in the efficiency of Zn absorption among plant species, and among different varieties within a species (Viets 1966; see Chapter 1.6.8).

In western regions, Zn deficiency occurs in irrigated soils used to grow crop species that are sensitive to Zn deficiency, but severely Zn deficient crops are usually confined to small areas within a field (Kubota and Allaway 1972). The eastern boundary for Zn deficient soils roughly corresponds to the eastern edge of the calcareous soil region (about the 100th meridian) of western USA.

In the southeast, Zn deficient soils are associated with sandy, well drained, acidic soils or with soils developed from phosphatic rock parent materials (e.g., parts of Kentucky and Tennessee). The Typic Quartzipsamments and associated soil series of the citrus-producing (Lakeland) region of Florida are the largest single area of Zn deficiency in the USA.

The area of slightly to moderately acidic Mollisol soils of the central region of the Corn Belt (i.e. in Iowa, Missouri, Illinois and Indiana) has a low incidence of Zn deficiency. Zinc deficiency in Pennsylvania, New Jersey, New York, and New England is common in susceptible crops in some local areas (Kubota and Allaway 1972).
1.2.2 Distribution of Zn deficient soils in Asia

Zinc deficiency often limits production of many crops throughout the tropics (Lopes 1980). Zinc deficient soils are found in the arid and semiarid regions rather than in the humid and sub-humid regions of India (Katyal and Vlek 1985). The north-central region of West Java, the tropical soils of Taiwan, and the poorly drained, calcareous paddy soils of China have been reported to be Zn-deficient (Katyal and Vlek 1985; Welch et al. 1991). In addition, grain yield increases to Zn fertilisers applied to rice soils in the Philippines have been reported (Katyal and Vlek 1985). Severe Zn deficiency of crops has also been reported in Sri Lanka (Katyal and Vlek 1985). Zinc deficiency of rice is commonly reported for crops growing in calcareous alkaline soils (Yoshida and Tanaka 1969). By contrast Bell et al. (1990a) found the soils of the semi-arid northeastern region of Thailand to be adequately supplied with Zn for food legume production.

1.2.3 Distribution of Zn deficient soils in Australia

In Australia, Zn deficient soils are widespread in parts of Western Australia (WA), Queensland, South Australia, Victoria, and New South Wales (Stephens and Donald 1958; Giles 1959, 1960; Anderson 1970; Donald and Prescott 1975). Zinc deficiency of crops and pasture plant species is found mainly in areas of acid sandy or gravelly lateritic soils formed over granitic gneiss, particularly in WA. Coastal soils formed from aeolian calcareous or leached siliceous sands over limestone are also Zn-deficient (Stephens and Donald 1958; Giles 1960; Donald and Prescott 1975; King and Alston 1975). The areas of grey and brown loams and sands in the western cereal growing district of WA (Gartrell and Glencross 1968, Gartrell 1969; Gilbey et al. 1970; Robson and Gilkes 1981) are severely Zn deficient for cereal and subterranean clover (Trifolium subteraneanum L.) (henceforth clover) production. In fact, WA has the distinction of having one of the largest single areas of Zn deficiency in the world (about 9 million ha) (Gartrell and
1.3 Zn in soils

1.3.1 Introduction

White (1957), Cox and Kamprath (1972), Nriagu (1980), Shuman (1980) and Tiller (1983) have reviewed Zn in soils. The availability of Zn to plants depends on several soil factors such as, the concentration of Zn in solution, ion speciation, and the interaction of Zn with other macronutrient and micronutrient elements (Hewitt 1963; Carroll 1967; Halvorson and Lindsay 1977; Robson and Pitman 1983; Shuman 1985a). The behaviour of Zn ions in soils and their uptake by plants cannot be explained by the total concentration of Zn in the soil. For example, the concentration of ZnOH$^+$ explained Zn adsorption on soil surfaces better than the total Zn concentration (Barrow 1986a). The availability of Zn to maize plants has been shown to depend on its form in solution (Halvorson and Lindsay 1977). Therefore, the speciation of Zn may be an important factor controlling Zn reactions in soil and its uptake by plants. The speciation of Zn (and other micronutrients) is controlled by the reactions that occur in soil solution while the concentration of Zn in solution is controlled by adsorption onto solid phases (Ellis and Knezek 1972; Singh and Sekhon 1977; Sidh et al. 1977).

1.3.2 Total concentration

Total Zn concentration of soils is largely dependent on the composition of the parent rock material (Graham 1953; Swaine 1955; Swaine and Mitchell 1960; Tiller 1963; Kabata-Pendias and Pendias 1984; Sillanpää 1972). In igneous rocks, low concentration of Zn is present in the sulfides, but higher concentration of Zn is in the more abundant silicates (Norrish 1975). The average concentration of Zn in basaltic rocks is usually higher than
the Zn concentration in granitic rocks. Magnetite is considered the most important Zn carrier in basaltic rocks, while biotite is the main source of Zn in granitic rocks (Wedepohl 1972). Mean Zn concentration ranges from 40 mg/kg in acid rocks (granites) to 100 mg/kg in basaltic rock (Krauskopf 1972). In sedimentary rocks, the highest Zn concentrations are found in shales and clayey sediments (80 to 120 mg/kg), while sandstones, limestones and dolomites generally have lower concentrations (10 to 30 mg/kg) (Kabata-Pendias and Pendias 1984).

The total Zn concentration of the lithosphere is about 80 mg Zn/kg, and the common range for soils is 10-300 mg Zn/kg, with an average concentration of 50 mg Zn/kg (Goldschmidt 1954; Krauskopf 1972; Wedepohl 1972). Tiller (1983) reported much higher and lower values that probably reflects the regions and countries from which the data was collected. Kabata-Pendias and Pendias (1984) reported values of 17 to 125 mg Zn/kg for numerous surface soils of different countries. The highest Zn concentrations tended to be found in some alluvial soils while concentration of Zn were lower for sandy textured soils and organic soils.

Total Zn concentrations are seldom used as a test for evaluating plant availability of soil Zn as the total Zn pool often incorporates Zn in unweathered minerals that is unavailable for plant growth.

1.3.3 Chemical behaviour of Zn in soils

Viets (1962) proposed 5 chemical pools for Zn in soils: (1) in soil solution, (2) on exchange sites of reactive soil components, (3) in complexes with organic matter, (4) occluded by or co-precipitated with oxides and hydroxides of Al, Fe and Mn and (5) held in primary and secondary minerals. These successive pools of Zn from ions in the soil solution to Zn in crystal lattices are considered to represent pools of decreasing availability for plant uptake (Viets 1962; Hodgson 1963). The importance of the pool
depends on the availability of Zn as well as the size of the pool. Pool 3 is generally considered the most importance source of Zn for plants as most soil Zn often occurs in this pool (Viets 1962; Hodgson 1963). The distribution of Zn among these forms is governed by the equilibrium constants of a series of possible soil reactions and processes that will be discussed in following sections.

Zinc in soils can be separated into fractions based on chemical analysis procedures and or particle size distribution. The amount of Zn in each of the chemical pools or forms varies due to the range of Zn concentration found in parent material of soils, and the extent of weathering processes. The various pools have been measured by several different sequential chemical extraction procedures (Rule and Graham 1976; Sedberry and Reddy 1976; Shuman 1979; Iyengar et al. 1981; Shuman 1985b; Singh et al. 1988; Beckett 1989; Novillo et al. 2002). The procedures differ in concentration and type of reagents and the methodology of extraction to reflect the nature of the soils being studied.

Table 1.1. The percentage distribution (%) of Zn in fractions of soils from a number of countries with varying cropping histories.

<table>
<thead>
<tr>
<th>Soil type</th>
<th>USA</th>
<th>USA</th>
<th>USA</th>
<th>USA</th>
<th>India</th>
<th>Australia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acidic(^a)</td>
<td>Acidic(^b)</td>
<td>Acidic(^c)</td>
<td>Acidic(^d)</td>
<td>Calcarious(^e)</td>
<td>Calcarious(^e)</td>
</tr>
<tr>
<td>Water soluble</td>
<td>2</td>
<td>0.4</td>
<td>0.3</td>
<td></td>
<td>&lt;0.25</td>
<td></td>
</tr>
<tr>
<td>Exchangeable</td>
<td>1</td>
<td></td>
<td></td>
<td>3</td>
<td>2</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Complexed</td>
<td></td>
<td></td>
<td>0.7</td>
<td>2</td>
<td>&lt;0.8</td>
<td></td>
</tr>
<tr>
<td>Carbonate</td>
<td>4</td>
<td>3</td>
<td>10</td>
<td>&lt;1</td>
<td>&lt;0.75</td>
<td></td>
</tr>
<tr>
<td>Organic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.75</td>
<td></td>
</tr>
<tr>
<td>Mn oxide</td>
<td>2</td>
<td></td>
<td>2</td>
<td>2</td>
<td>&lt;0.75</td>
<td></td>
</tr>
<tr>
<td>Al and Fe oxide</td>
<td>25</td>
<td>2</td>
<td>17</td>
<td>12</td>
<td>15.0</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>86</td>
<td>70</td>
<td>97</td>
<td>67</td>
<td>82</td>
<td>80.0</td>
</tr>
</tbody>
</table>

\(^a\) Sedberry and Reddy (1976).
\(^b\) Iyengar et al. (1981).
\(^c\) Mandal and Mandal (1986).
\(^d\) Shuman (1985b).
\(^e\) Singh et al. (1988).
\(^f\) Ma and Uren (1997).

The forms of Zn usually measured are water soluble, exchangeable, carbonate bound,
organic matter bound, Fe and Mn oxide bound and residual fractions (see Table 1.1). The varying fractionation procedures make it difficult to directly compare results, and suggest the need for a more universal method for assessing the forms of Zn in soils.

Another method of fractionation of soil Zn is to segregate the soil into sand, silt and clay (Genrich and Bremner 1972, 1974) before using chemical reagent extraction techniques. The methods and procedures (for example, Genrich and Bremner 1972, 1974) used to segregate soil into particle sizes could possibly alter the chemistry of Zn in the soil. However, dispersion techniques of segregating the soil into sand, silt and clay minimise the change in the chemistry of the soil, hence minimising possible effects on the soil chemistry of Zn (Khan 1979). Generally, the highest concentrations of DTPA-extractable Zn for 15 United States soils with texture ranging from sands to clays were found in the clay and the lowest in the sand fraction (Lindsay and Norvell 1969). The percentage soil DTPA Zn in clay was 66 % while 20 % was found in the silt fractions of the soil. Most chemical fractionation procedures show that residual Zn is the greatest component of soil Zn (see Table 1.1). Ma and Uren (1997) found that recently added Zn was found in the EDTA extractable fraction, and then transformed to the iron oxide fraction with time. Ma and Uren (1997) found that wetting, drying and elevated soil temperature enhanced the transformation of recently added Zn to Fe (Al) and Mn oxide fractions, although the rates of transformation were not studied. Novillo et al. (2002) showed that no Zn was detectable in the water soluble plus exchangeable fraction in a calcareous soil. However, Novillo et al. (2002) showed that some Zn remained in labile fractions of acidic and neutral soils. Consequently, the percentage of Zn in labile or exchangeable form is small but it would appear that roots of plants obtain Zn from these pools (Ma and Uren 1997; Novillo et al. 2002). Although these extraction procedures are commonly used there is little direct evidence that plant roots can differentiate among the various pools of soil Zn in acquiring soil Zn (Brennan et al. 1993).
Chemical reactions in soil solution never reach their equilibria (Lindsay 1972b), as the soil system is an open and dynamic system with additions and losses of ions continually taking place. Changing moisture concentration in the soil, pH changes, mineralisation of organic matter, plant root exudates and changing redox status of the soil will result in a shift of the chemical equilibrium and a transfer of Zn from one form to another.

1.3.4 Soil solution Zn

Zinc in the soil solution may occur as $\text{Zn}^{2+}$, $\text{ZnCl}^+$, and $\text{ZnOH}^+$, complexed with organic matter (organic ligands) (as discussed in Section 1.3.5) or associated with colloidal particles. The extent of speciation of Zn depends on the stability constants for the species formed, ionic strength, pH and the type and relative concentrations of cations and anions present in solution (Lindsay 1972b).

The stability constant ($K$) is defined as

$$K = \frac{(M_aL_b)^{a-b}}{(M^{x+})^a (L^{y-})^b}$$

from the equation $aM^{x+} + bL^{y-} = M_aL_b^{a-b}$

where $M = \text{metal ion}$, $L = \text{ligand}$, $a = \text{moles of metal ions}$, $b = \text{moles of ligand molecules}$ (Stevenson and Ardakani 1972).

A relationship between Zn concentration and pH has been reported for a small group of United States soils: $[\text{Zn}^{2+}] = [\text{H}^+]^2 \times 10^6$ (Lindsay 1972b).

Sanders (1983) found that the concentration of Zn in soil solution from a sandy loam decreased with pH from 163 $\mu$g/L at pH 4.8 to 5 $\mu$g/L at pH 6.5. $\text{Zn}^{2+}$ was the dominant species in a lateritic podzolic soil over a pH range of 3.8 to 5.6 (Chairidchai and Ritchie 1990). Although $\text{ZnOH}^+$ (and $\text{ZnCl}^+$) occurred at lower concentration compared with $\text{Zn}^{2+}$, the concentration of $\text{ZnOH}^+$ increased by nearly 100 fold over the 3.8 to 5.6 pH range (Chairidchai and Ritchie 1990). Similarly, McGrath et al. (1988) found that 15 to
30% of Zn in soil solution of a sandy loam was in the Zn$^{2+}$ form. Jeffrey and Uren (1983) reported that the Zn concentration in soil solution decreased from 1800 to 12 µg/L with an increase in pH from 4.4 to 7.5. The proportion of Zn that was labile varied from >99% at pH 4.4 to about 22% at pH 7.5. The lability of Zn was defined as the ability of metal ions to dissociate from their various ligands.

Table 1.2. Solubility of a range of Zn minerals, equilibrium reactions and log $K^\circ$ values.

<table>
<thead>
<tr>
<th>Equilibrium reaction$^a$</th>
<th>log $K^\circ$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxides and hydroxides</strong></td>
<td></td>
</tr>
<tr>
<td>1 Zn(OH)$_2$ (amorph) + 2H$^+$=Zn$^{2+}$ + 2H$_2$O</td>
<td>12.48</td>
</tr>
<tr>
<td>2 α-Zn (OH)$_2$(C) + 2H$^+$=Zn$^{2+}$ + 2H$_2$O</td>
<td>12.19</td>
</tr>
<tr>
<td>3 β-Zn(OH)$_2$(C) + 2H$^+$= Zn$^{2+}$ + 2H$_2$O</td>
<td>11.78</td>
</tr>
<tr>
<td>4 Y-Zn (OH)$_2$(C) + 2H$^+$=Zn$^{2+}$ + 2H$_2$O</td>
<td>11.74</td>
</tr>
<tr>
<td>5 c-Zn(OH)$_2$(C) + 2H$^+$= Zn$^{2+}$ + 2H$_2$O</td>
<td>11.53</td>
</tr>
<tr>
<td>6 ZnO (zincite) + 2H$^+$=Zn$^{2+}$ + H$_2$O</td>
<td>11.16</td>
</tr>
<tr>
<td><strong>Carbonates</strong></td>
<td></td>
</tr>
<tr>
<td>7 ZnCO$_3$ (smithsonite) + 2H$^+$ = Zn$^{2+}$ + CO$_2$(g) + H$_2$O</td>
<td>7.91</td>
</tr>
<tr>
<td><strong>Soil-Zn and Zn-Fe oxide</strong></td>
<td></td>
</tr>
<tr>
<td>8 ZnFe$_2$O$_4$ (franklinite) + 8H$^+$=Zn$^{2+}$ + 2Fe$^{3+}$ + 4H$_2$O</td>
<td>9.85</td>
</tr>
<tr>
<td>9 Soil-Zn + 2H$^+$ = Zn$^{2+}$</td>
<td>5.8</td>
</tr>
<tr>
<td><strong>Silicates</strong></td>
<td></td>
</tr>
<tr>
<td>10 Zn$_2$SiO$_4$ (willemite) + 4H$^+$ = 2Zn$^{2+}$ + H$_2$SiO$_4$</td>
<td>13.15</td>
</tr>
<tr>
<td><strong>Chlorides</strong></td>
<td></td>
</tr>
<tr>
<td>11 ZnCl$_2$(C) = Zn$^{2+}$ + 2 Cl$^-$</td>
<td>7.07</td>
</tr>
<tr>
<td><strong>Sulfates</strong></td>
<td></td>
</tr>
<tr>
<td>12 ZnO - 2ZnSO$_4$(C) + 2H$^+$= 3Zn$^{2+}$ + 2SO$_4^{2-}$ + H$_2$O</td>
<td>19.12</td>
</tr>
<tr>
<td>13 Zn (OH)$_2$. ZnSO$_4$(C) + 2H$^+$= 2Zn$^{2+}$ + SO$_4^{2-}$ + 2H$_2$O</td>
<td>7.5</td>
</tr>
<tr>
<td>14 ZnSO$_4$ (zinkosite) = Zn$^{2+}$ + SO$_4^{2-}$</td>
<td>3.41</td>
</tr>
<tr>
<td><strong>Phosphates</strong></td>
<td></td>
</tr>
<tr>
<td>15 Zn$_3$(PO$_4$)$_2$.4H$_2$O(hopeite) + 4H$^+$ = Zn$^{2+}$ + 2H$_2$PO + 4H$_2$O</td>
<td>3.8</td>
</tr>
</tbody>
</table>

$^a$Lindsay 1972b, 1979
Hodgson *et al.* (1965, 1966) showed that an average of 60% of Zn in soil solution was complexed by organic compounds and values for the Zn concentration in soil solution ranging from $3 \times 10^{-8}$ to $3 \times 10^{-6}$ M, extremely low values compared to the average total Zn concentration of soils (50 mg/kg) for 20 calcareous soils from Colorado. Kabata-Pendias and Pendias (1984) reported soil Zn solution values ranging from 4 to 270 µg/l (about $6 \times 10^{-8}$ to $4 \times 10^{-6}$ M), depending on the soil type and the extraction technique used for obtaining the soil solution. More recent research has indicated that Zn$^{2+}$ activity in soil solution was much lower than reported concentrations but varied over a wide range from approximately $1 \times 10^{-12}$ to $2.2 \times 10^{-10}$ (Norvell and Welch 1993; Parker 1993; Welch and Norvell 1993).

The Zn$^{2+}$ ion in soil solution will precipitate when the solubility product of a compound with any reactive anion is exceeded. Insoluble Zn compounds of hydroxide, carbonate, phosphate, sulfide, and molybdate and with several organic ligands, including humates and fulvates may form in soil systems. Lindsay (1972b, 1979) proposed several theoretical equations on the possible chemical activity of Zn ions in soils based on the solubility products of the different Zn compounds (see Table 1.2). Reaction 9 in Table 1.2 gives the solubility of soil Zn based on experimental measurement for a number of soils (Norvell and Lindsay 1969, 1970; Lindsay 1972b, 1979; Norvell 1972; Catlett *et al.* 2002). The equilibrium constant for this reaction,

$$\text{Soil-Zn} + 2\text{H}^+ = \text{Zn}^{2+} \quad \log K^o = 5.8$$

may be expressed as follows:

$$\log \text{Zn}^{2+} = 5.8 - 2\text{pH} \quad \text{or} \quad p\text{Zn} = 5.8 - 2\text{pH}$$

This equation provides a useful benchmark for estimating solubility for Zn$^{2+}$ in soils. It shows that the activity of Zn$^{2+}$ in the soil solution is directly proportional to the square of
the proton activity. This means that the solubility of Zn will increase at decreasing pH values of the soil. Catlett et al. (2002) found for 18 Colorado soils that the log of the Zn$^{2+}$ concentration was significantly related to the soil organic carbon and the soil pH value.

Fotovat et al. (1997) demonstrated that Zn minerals, for which solubility data are available, were too soluble to control the solubility of Zn in soil solution of eight diverse soils of South Australia. Although Lindsay (1979) suggested that ZnFe$_2$O$_4$ (franklinite) may govern the solubility in acid soils, the study of Fotovat et al. (1997) showed that the soil solution Zn$^{2+}$ concentration was less than predicted by relationship 9, (Table 1.2) of Lindsay (1979). Gilmour and Kittrick (1979) suggested that ZnS (sphalerite) may govern Zn concentration in the soil solution, but it was not shown to do so for the eight soils of South Australia (Fotovat et al. 1997). Generally Zn concentration tended to be higher in alkaline soils than that predicted from the solubility constants for Zn minerals, which may suggest that soluble-organic ligand complexation was underestimated by the log Zn$^{2+}$-pH relationship of Lindsay (1979). It has been suggested that Zn solubility at high soil pH is due to organic matter dispersion which can either release complexed Zn or supply ligands for metal complexation and decrease precipitation (Saeed and Fox 1977).

1.3.5 Sorption of Zn

**Introduction:** The term adsorption is commonly used for the processes of sorption of chemical elements from solutions at the surface of soil particles (Kabata-Pendias and Pendias 1984). The term sorption refers to all phenomena at the solid-solution boundary that may include adsorption and precipitation. The important soil components for the adsorption of Zn are clay minerals, hydrated metal oxides and organic matter; the colloidal phase of the soil. At normal soil pH values, the surface of the colloidal phase has a net negative charge. The negatively charged adsorption sites are compensated by equivalent amounts of positive charges, such as protons and other cations, such as Zn$^{2+}$. 

Therefore, the adsorption of Zn from the soil solution by the solid soil particles is generally accompanied by the simultaneous desorption of equivalent amounts of other cations from the solid phase to the soil solution. This process is called ion exchange or equivalent adsorption.

The adsorption of Zn by soils and soil constituents has shown that clays and organic matter adsorb Zn quite strongly (Benson 1966; Trehan and Sekhon 1970; Lindsay 1972b; Farrah and Pickering 1976; Wada and Adb-Elfattah 1978; McBride 1989). Tiller (1983) suggested that for Australian soils the concentration of Zn in solution is controlled largely by Zn adsorption.

*Adsorption and desorption of Zn in soils:* Pickering (1980) and Shuman (1980) have reviewed the processes of Zn adsorption in soil. Jurinak and Bauer (1956) showed that Zn is more strongly adsorbed on clay minerals at alkaline pH values. Shuman (1975) showed that soils high in clay or organic matter had higher Zn adsorption than more sandy soils (lower clay content). Abd-Elfattah and Wada (1981) found the highest adsorption of Zn by Fe oxides, halloysite and allophanes and the lowest by montmorillonite. Thus, clay minerals, hydrous oxides, organic matter and pH are important factors affecting Zn adsorption by soils (Stanton and Burger 1967, 1970; Bar-Yosef 1979; Barrow 1993; Barrow and Whelan 1998).

Zinc was found to be adsorbed reversibly by ion-exchange and irreversibly into lattice structures of clay minerals (Elgabaly 1950; Tiller and Hodgson 1962). Montmorillonite, especially at neutral or alkaline pH values, sorbed Zn in amounts in excess of the cation exchange capacity (CEC) (De Mumbrum and Jackson 1956; Bingham *et al.* 1964). The adsorption of ZnOH\(^+\) and or the precipitation of Zn(OH)\(_2\) can explain the apparent excess sorption. Zinc, like other heavy metal ions, is strongly hydrolyzed (ZnOH\(^+\)), as it is able to form strong bonds with oxygen atoms (James and Healy 1972; Barrow 1986b, 1987).
The hydrolysis reaction may be particularly important in determining the adsorption of the metal ions onto solid surfaces (James and Healy 1972; Barrow 1986b). Chairidchai and Ritchie (1990) also reported that Zn adsorption was well correlated ($R^2 = 0.90$) with the concentration of ZnOH$^+$ in the final soil solution but not correlated with other Zn species. Therefore, ZnOH$^+$ is an important chemical species in soil solution for chemical reactions (adsorption) and plant uptake.

McBride and Blasiak (1979) reported that nucleation of Zn hydroxide on clay surfaces may produce strongly pH dependent retention of Zn in soils. Reddy and Perkins (1974) studied the adsorption of Zn by bentonite, illite and kaolinite at different pH values and found that Zn was precipitated in clay lattice zones or adsorbed at surface exchange sites.

Quirk and Posner (1975) suggested that adsorption-desorption reactions usually controlled the availability of Zn (Cu, Co, Mo and B) in soils. Brummer et al. (1983) demonstrated that the Zn concentrations in the soil solution are controlled by adsorption-desorption reactions. Empirical equations, such as the Langmuir adsorption equation have successfully described the adsorption of Zn by soils (Udo et al. 1970; Shuman 1975; Sinha et al. 1975). Zinc availability was then discussed in terms of adsorption-desorption reactions instead of by an unknown soil-Zn compound. The solubility product principle or precipitation dissolution reactions were important only at alkaline pH values and high equilibrium Zn concentrations. Under these conditions, the precipitation-dissolution reactions dominate over adsorption-desorption processes and specific Zn compounds like Zn phosphate may form (Kalbasi et al. 1978). Other possible compounds that may be important for precipitation-dissolution reactions are Zn carbonate (Misra and Tiwari 1966; Udo et al. 1970; Kuo and Mikkelsen 1979) and Zn silicate (Tiller and Pickering 1974).

Specific and non-specific sorption reactions can be separated by use of appropriate electrolyte in excessive amounts to effectively reduce non-specific sorption reactions and
by carrying out reactions under conditions where the surface and absorbate have the same charge (Tiller 1983). Iyengar et al. (1981) separated exchangeable Zn in calcareous soils into non-specifically adsorbed and specifically adsorbed Zn.

Specifically adsorbed Zn: Zinc is said to be specifically adsorbed because the ion is sorbed by surfaces of synthetic oxides of Fe and Al (goethite, gibbsite) that have a net positive charge, so despite electrostatic repulsion, Zn is still adsorbed in significant amounts (Forbes et al. 1976; Bolland et al. 1977). That is specific adsorption may overcome repulsion forces arising when the ion and the adsorption surface have the same electrical charge. Both cations and anions (anions: Hingston et al. 1967; cations: Forbes 1973; Quirk and Posner 1975) can be specifically adsorbed. Examples of cations capable of specific adsorption are Zn, Cu and Co (Forbes 1973; Quirk and Posner 1975). Specifically adsorbed Zn can only be replaced by cations with similar affinities or greater for the absorption surface or by chemical extraction with chelating agents (Quirk and Posner 1975).

The specific adsorption of Zn onto oxides of Fe, Mn and Al has been extensively studied (Mitchell 1964; Jenne 1968; McKenzie 1972; Gadde and Laitinen 1974; Kinniburgh and Jackson 1974; Forbes et al. 1976; Kinniburgh et al. 1976; Shuman 1976; Bolland et al. 1977; Loganathan et al. 1977; Parfitt and Russell 1977; Shuman 1977; Kalbasi et al. 1978; Rudgers 1978; Bowden et al. 1980, Farrah et al. 1980; McKenzie 1980; Gerth and Brummer 1981; Padmanabham 1983; Tiller et al. 1984; Ghanem and Mikkelsen 1988). Jenne (1968) and Loganathan et al. (1977) proposed that hydrous oxides of Mn and Fe play a major role in the adsorption of Zn (and Co, Ni and Cu) in soils. The oxides of Mn and Fe that have high surface area have wide distribution in Australian soils (McKenzie 1972; McKenzie 1980; Taylor et al. 1983). The most common Fe oxides found in soil are goethite, lepidocrocite, hematite, maghemite, ferrihydrite and magnetite. Taylor et al.
(1983) listed 13 manganese oxides that are found in soils of which birnessite and lithiophorite are the most common. In most agricultural soils, Mn oxides have a negative surface charge and a high affinity for Zn (Taylor and McKenzie 1966; McKenzie 1972; Zosaski and Burau 1975). Gibbsite and boehmite are the most common aluminium oxides in soils. Reactions of most cations with aluminium oxides are considered to be similar to those with Fe oxides (Taylor et al. 1983).

Specific adsorption of Zn (and Cu) onto goethite may occur at a pH below the point of zero charge (PZC) (Sposito 1981) of the surface so that there is adsorption of positive ions onto a positive surface. The PZC of the adsorbate and pH have major effects upon the rate of adsorption of Zn onto oxides (Kalbasi et al. 1978). The rate of adsorption of Zn onto goethite was slow at low pH but increased rapidly with increasing pH due to the high PZC of the goethite (7.6). Amounts of Zn adsorbed onto goethite also increased over a pH range of about 4.2 to 6.1 (Padmanabham 1983).

Zinc adsorption onto Mn and Fe oxides increased with pH (Loganathan et al. 1977). Manganese oxides had a lower PZC (1.8 to 3.5 for a range of Mn oxides) and adsorption was greater at lower pH values (McKenzie 1980). However, there were differences in both the quantities of Zn adsorbed and the response to changes in pH between Mn and Fe oxides (McKenzie 1980). Adsorption of Zn by Mn oxide (birnessite) was near linear with pH from 3 to 7 while for the Fe oxides (hematite and goethite) the adsorption increased exponentially.

Kalbasi et al. (1978) reported that adsorption of Zn by Al oxide was pH dependent and increased markedly with increasing pH. At equivalent pH values, Fe oxides adsorbed more Zn than Al oxides.

**Influence of soil organic matter on the behaviour of Zn in soils;** Soil organic matter is an important soil constituent, consisting of a range of organic compounds such as humic
substances, organic acids of low and high molecular weight, carbohydrates, proteins, peptides, amino acids, lipids, waxes, polycyclic aromatic hydrocarbons and lignin fragments (Stevenson 1967; Stevenson and Ardakani 1972). Many of these components of soil organic matter have a strong affinity to bind Zn (Yortensen 1963; Randhawa and Broadbent 1965a, b; Schnitzer and Skinner 1966; Schnitzer and Khan 1978).

The most stable organic compounds in soils are humic substances; these can be divided into humic acids and fulvic acids (Stevenson 1991). Humic acids are soluble in alkaline media, and generally have higher molecular weights than fulvic acids which are soluble in both alkaline and acid medium. Both humic and fulvic acids substances contain a large number of functional groups (OH, COOH, SH, C=O) that have a great affinity for Zn and other micronutrient ions (Cu, Mn) (Stevenson 1991).

Reactions with organic matter have an effect on most aspects of Zn chemistry in soils and have been reviewed (Stevenson and Ardakani 1972; Stevenson 1991). Zinc complexed with insoluble humus complexes is a significant component of the specifically adsorbed Zn while additional Zn is non-specifically adsorbed on exchange sites of organic matter (Himes and Barber 1957).

Stevenson and Ardakani (1972) divided organic compounds that form stable complexes in soil into 2 groups: (1) simple compounds such as organic acids, polyphenols, amino acids, peptides, proteins and polysaccharides, and (2) complex polymers formed by secondary synthesis reactions such as humic and fulvic acids. Soluble Zn complexes are mostly from group 1 and these maintain Zn in solution at soil pH values that would otherwise result in insoluble precipitates of Zn compounds. However, reactions of Zn with the second group tend to form insoluble complexes that are unavailable to plants, although humic and fulvic acids may also form soluble complexes (Stevenson and Ardakani 1972). The relative significance of these compounds is difficult to determine because of
analytical problems in measuring the small quantities involved, their dynamic nature due to the balance between synthesis and degradation by micro-organisms and between sorption and desorption, and their heterogeneous distribution throughout the soil (Stevenson and Ardakani 1972). However, Hodgson et al. (1966) and Geering and Hodgson (1969) found that 60-75 % of soluble Zn was present as soluble organic complexes.

Many authors (Mortensen 1963; Hodgson 1963; Yortensen 1963; Norvell 1972; Stevenson and Ardakani 1972; McBride 1978, 1989; Norvell 1991) have studied the interactions between humic substances and Zn. Fulvic acids form chelates with Zn ions over a wide pH range, thus increasing the solubility and mobility of Zn. Schnitzer and Skinner (1966) and Stevenson and Ardakani (1972) determined the stability constant (log K) of Zn fulvate as 1.7 at pH 3.5 and 2.3 at pH 5. Schnitzer and Khan (1978) reported log K values of 2.3 at pH 3 and 3.6 at pH 5. Thus, the Zn-complex stability increases with increasing pH value in the acid range. However, Alloway (1990) reported that two stability maxima occurred, one at about pH 6 and another at pH 9 which was attributed to the dissociation of carboxyl and hydroxyl functional groups, respectively in the fulvic acid molecule. Furthermore, Zn fulvates show only weak colloidal properties, which means that flocculation of fulvic acids occurs only at high electrolyte concentrations. Therefore, fulvic acids act in soils as mobilising agents for Zn (and Cu, Fe and Pb).

Humic acids are insoluble in acid conditions, and dissolve gradually as pH increases. In alkaline media, humic acids are completely soluble, but behave as a colloidal system, which means that they can be flocculated by cations (Ca and Mg are often the main flocculating cations). Alloway (1990) discussed the behaviour of Zn in purified humic acid-Zn systems. At low pH values most of the Zn was present as cations because the humic acid was insoluble, however, humate complexes with Zn were formed as soil pH
values increased. Most of the Zn humates were soluble, and at alkaline pH values only a small fraction of Zn was present as Zn hydroxides (Alloway 1990). Therefore, soil organic matter is an important factor affecting the behaviour of Zn in soils. The fulvic acids and low molecular weight organic acids mainly form soluble complexes and chelates with Zn, thus increasing its mobility in the soil.

**Weakly available Zn**: Zinc minerals, precipitates and occluded forms of Zn are least available to plants. The main minerals of Zn are ZnS (sphalerite), ZnO (zincite), ZnCO$_3$ (smithsonite) and Zn silicates (Krausopf 1972; Norrish 1975). Lindsay (1972b) suggested that pure minerals are too soluble to persist in soil and hence cannot control the solubility of Zn in soil. Zinc can undergo isomorphous substitution for Mg and Fe in silicate minerals and may be occluded in minerals such as oxides and hydrous oxides of Fe, Al and Mn and in carbonates (Lindsay 1972b; Tiller 1983). Non-exchangeable Zn associated with these compounds is stable and not immediately available to the plant (Cox and Kamprath 1972).

1.4 Zn in plants

1.4.1 Introduction

Various reviews on Zn nutrition of plants are available that cover the role and function of Zn in plants (Camp and Fudge 1945; Chapman 1966; Viets 1966; Anderson 1972; Epstein 1972; Lindsay 1972a; Mengel and Kirkby 1978; Marschner 1993; Brown *et al.* 1993).

Crop species sensitive to Zn deficiency are the cereals (wheat and barley), maize, sorghum, flax, hops, cotton, legumes (particularly subterranean clover), grapes, citrus and many fruit tree species (Chapman 1966; Wallace 1966; Bould *et al.* 1983). Typical names for Zn deficiency are white bud (corn and sorghum), little leaf (fruit trees), mottle leaf
The Zn concentration of plants varies with different soils, climatic factors and with plant genotypes (see review of Jones 1991). Zinc concentration (mg Zn/kg) in mature leaves can generally be classified as follows: (i) deficient, if <10 to 20, (ii) sufficient or normal if between 25 and 150, (iii) excessive or toxic if >400 (Kabata-Pendias and Pendias 1984). Zinc levels in various plant species and for a range of plant parts (e.g. whole shoots, youngest emerged leaves, and youngest mature leaves) have been compiled by various authors (Chapman 1966; Bergmann 1983; Snowball and Robson 1983; Grundon 1987; Weir and Creswell 1994; Reuter and Robinson 1997).

1.4.2 The absorption of Zn by roots

The processes involved in the absorption of ions by roots have been reviewed (Rains et al. 1964; Laties 1969; Pitman 1972; Epstein 1972; Moore 1972; Pitman 1976; Kochian 1991; 1993; Reid et al. 1996; Williams et al. 2000; Sattelmacher 2001).

$\text{Zn}^{2+}$ seems to be the main ionic species absorbed by plant roots, but hydrated Zn and several other complexes and Zn organic chelates may also be absorbed (Loneragan 1975; Kabata-Pendias and Pendias 1984; Pulford 1986; Bell et al. 1991; Marschner 1993).

Zinc absorption has been demonstrated to be a metabolically mediated process in a range of tissues but differing conclusions have been reached over whether Zn uptake is an active process or passive process. Zinc absorption by excised barley roots was depressed by anaerobic conditions, lowering temperatures and by metabolic uncouplers (Schmidt et al. 1965). Bowen (1969) also demonstrated that Zn absorption by sugar cane leaves was severely depressed by inhibiting oxidative phosphorylation. The $Q_{10}$ values for the absorption of Zn by wheat roots were greater than unity between 2º C and 29º C (Chaudhry and Loneragan 1972a). Bowen et al. (1974) found that low temperatures...
depressed the absorption of Zn by roots of *Pinus radiata*. These observations suggest that the absorption of Zn by plant roots was an active process.

In contrast, Rathmore *et al.* (1970) concluded that Zn absorption was a passive process. However, as the plant tissues of the Rathmore *et al.* (1970) study were not desorbed, the estimates of Zn absorption probably included a large adsorption component causing some doubts about the conclusions. Other reports have suggested that Zn$^{2+}$ uptake is not metabolically–dependent due to the lack of response to metabolic inhibitors (Broda *et al.* 1964; Gutknecht 1961, 1963).

Absorption of Zn by plant roots has been reviewed by Kochian (1993). Kochian (1993) suggested that the phytosiderophores (low molecular weight molecules), which complex iron and are released by plant roots, are involved in Zn$^{2+}$ absorption in grasses. For the dicots and monocots (excluding grasses) the influx of Zn$^{2+}$ into root cells has been suggested to be via a divalent cation channel, with the driving force due to a negative membrane potential. This passive transport system is still coupled to metabolism, and the use of metabolic inhibitors would result in an inhibition of uptake due to a reduction of the membrane potential. Kochian (1993) speculated that the cation channel could also explain the competitive uptake of Cu$^{2+}$ (hydrated radii = 0.42 nm) and Zn$^{2+}$ (hydrated radii = 0.43 nm) without the need for an active transport system. Similarly, Kochian (1993) suggested that several recent technical advances, such as chelate buffer techniques, patch clamp and other microelectrode approaches would better enable researchers to understand how plants absorb and transport Zn (and other mineral elements). Although the existence of a cation channel system can explain the various observed phenoma (contrasting metabolic or passive process, and Cu$^{2+}$/Zn$^{2+}$ competitive uptake) of Zn absorption, it awaits verification.

Zinc may move through the root to the xylem either via the cytoplasmic continuum of
root cells linked by the plasmodesmata (symplast) or via the extracellular spaces between the cells (apoplast). Recently, Lasat and Kochian (2000) presented a schematic model of Zn fluxes across cell membranes to account for the hyperaccumulation of Zn in *Thlaspi caerulescens*. The model contrasted the magnitude of the Zn fluxes across cell membranes of *Thlaspi caerulescens* with those of *Thlaspi arvense*, a non-accumulator of Zn. It was proposed that the entry point for Zn was across the plasma membrane and that all Zn reached the xylem via the symplastic pathway for *T. caerulescens* and *T. arvense* (Lasat *et al.* 1996; Lasat and Kochian 2000). However, Zn may also reach the xylem via an apoplastic pathway. White *et al.* (2002) concluded that with increasing external Zn concentrations the apoplastic pathway contributes to the uptake of Zn and influx to the xylem. Future studies would need to determine the relative contribution of the symplastic and apoplastic pathways in the uptake of Zn to the xylem of roots especially at concentrations more typical of agricultural soils.

### 1.4.3 Factors affecting plant Zn uptake

**Effect of Nitrogen:** Addition of N can influence the response of plants to Zn by: (i) the effects of N supply on dilution of Zn within plants, and (ii) the effect of N supply on the root/shoot ratio (Ozanne 1955a; Viets *et al.* 1957).

At low levels of soil Zn, high amounts of N applied can induce Zn deficiency by increasing plant growth to such an extent that absorbed Zn is diluted within the plant to deficient concentrations (Terman *et al.* 1966; Chaudhry and Loneragan 1970; Soliman *et al.* 1979). That is, the N application has increased plant growth but decreased Zn concentration in shoots often without decreasing the Zn content (product of plant shoot weight and its Zn concentration) of plants.

Nitrogen may also affect the response of plants to Zn by decreasing the root to shoot ratio (Reuthers and Smith 1950; Chaudhry and Loneragan 1970). Any factor, which increases
plant growth without concomitantly increasing the rate of absorption or the size of the root system, will result in a decrease in concentration of Zn in the plant (Loneragan 1975). Nitrogen application to soil deficient in N stimulates shoot growth to a greater extent than promoting root growth, thus decreasing the root to shoot ratio.

**Alkaline earth cations:** In solution studies, cations such as the alkaline earth cations (Ca$^{2+}$, Mg$^{2+}$, Sr$^{2+}$, Ba$^{2+}$), K$^+$ and NH$_4^+$ inhibit Zn absorption by plants at levels which are not in themselves toxic (Chaudhry and Loneragan 1972a, b, c; Loneragan and Webb 1993). The macronutrient cations all inhibited the rate of Zn absorption strongly in solutions of low Ca concentrations (0 to 40 mM). Increasing the Ca concentration to 100 mM, lessened inhibitory effects and for K$^+$ and Mg$^{2+}$ the effects eventually disappeared. Chaudhry and Loneragan (1972 a, b, c) suggested the inhibition and progressive lessening of the effect of macronutrients on the rate of Zn absorption by increasing Ca concentrations indicated a non-competitive mechanism. Further, kinetic analysis has indicated that the alkaline earth cations non-competitively inhibit Zn$^{2+}$ uptake and do not share the same transport system.

**Micronutrients:** Micronutrient cations, particularly Cu$^{2+}$ have been reported to have toxic and inhibitory effects that may influence the uptake of Zn by plants (Dunne 1956; Olsen 1972; Chaudhry *et al.* 1973; Brar and Sekhon 1976; Loneragan and Webb 1993). At high concentrations of micronutrients the effects are mainly toxic whereas at low concentrations the effect is an inhibition of Zn uptake. The inhibition of Zn absorption by Cu has been established in solution culture studies (Schmidt *et al.* 1965; Hawf and Schmidt 1967; Bowen 1969; Chaudhry and Loneragan 1972b; Giordano *et al.* 1974). The inhibitory effect has been found to be due to the competition between Cu$^{2+}$ and Zn$^{2+}$ for absorption sites in the plasma membrane of roots (Chaudhry *et al.* 1973).

Similarly, the inhibition of Zn absorption by Cu has been established in soils in glasshouse and field studies (Dunne 1956; Toms 1958; Gartrell 1969; Kauser *et al.* 1976).
A strong Cu-Zn interaction has been observed in the grain yield of wheat grown in soils deficient in Cu and Zn in WA (Toms 1958; Chaudhry and Loneragan 1970). In these soil studies, Cu did not depress absorption of Zn, but by contrast Zn severely depressed Cu uptake by the plant. The apparent conflict between the soil and the solution studies may be due to the effects of complexation with organic ligands on the activities of Cu$^{2+}$ and Zn$^{2+}$ ions that are the dominant ions absorbed (Kochian 1991). In solution studies, the Cu and Zn are present as divalent ions while in most soils these ions are complexed, with a greater proportion of Cu complexed than Zn (Hodgson et al. 1965, 1966; Geering and Hodgson 1969; Norvell and Lindsay 1972).

The interaction between Fe and Zn is complex and there appears to be many conflicting results. Increasing Fe supply has generally had a depressive effect on Zn concentration in plant shoots (Watanabe et al. 1965; Zhang et al. 1991a). However, increasing Fe supply has been shown to increase (Giordano et al. 1974), have no effect (Chaudhry and Loneragan 1972b) or decrease (Giordano et al. 1974; Rashid et al. 1976; Zhang et al. 1991b) the concentration of Zn in plant shoots. The conflicting evidence is probably due to differences in experimental conditions, plant species, solution composition and/or complexation of the Fe, and release of root exudates mobilising iron (Zhang et al. 1989). The Fe-Zn inhibitory interaction is unlikely to affect crop yields in cropping systems of WA where Fe fertiliser is not applied to broadscale agricultural crops (Brennan and Highman 2001).

**Anion effects:** Anions affect Zn uptake by plants by a variety of mechanisms. The important anions that will be considered here are phosphate (PO$_4^{3-}$), nitrate (NO$_3^{-}$) and organic ligands.

**The phosphate effect:** The interaction of PO$_4^{3-}$ and Zn$^{2+}$ for a range of plant species and soil types has been studied since about 1936 (Barnette et al. 1936; Boawn et al. 1954,
1957; Thorne 1957; Burleson et al. 1961; Langin et al. 1962; Stukenholtz et al. 1966; Boawn and Leggett 1964; Terman et al. 1966; Boawn and Brown 1968; Brown et al. 1970; Terman et al. 1972; Farah and Soliman 1986; Singh et al. 1986; Soliman and Farrah 1987; Parker et al. 1992) and the subject has been reviewed extensively (Olsen 1972; Shuman 1980; Robson and Pitman 1983; Webb and Loneragan 1988; Loneragan and Webb 1993). The interaction, often called "P-induced Zn deficiency" is commonly associated with high levels of available soil P or with application of P to the soil. Applying various sources of Zn to the soil has prevented or corrected the symptoms of Zn deficiency (Olsen 1972; Loneragan et al. 1979). Although abundant literature on the P-Zn interaction is available the subject remains complex as many phenomena in both soil and plants are involved.

Several possible explanations for the P/Zn effect have been proposed (Dwivedi et al. 1975; Welch et al. 1982; Cakmak and Marschner 1986; Loneragan and Webb 1993). Possible mechanisms for the P/Zn mechanism have included a range of plant factors; (i) the dilution of Zn concentration in the plant due to P supply increasing growth (Loneragan 1951; Bingham and Garber 1960; Watanabe et al. 1965; Sharma et al. 1968a, b), (ii) P interfering with the translocation and utilisation of Zn within the plant (Thorne 1957; Millikan et al. 1968; Warnock 1970; Adriano et al. 1971; Khan and Zende 1977; Marschner and Cakmak 1986), (iii) decreased absorption of Zn by plants (Bingham and Garber 1960; Stukenholtz et al. 1966; Cogliatti et al. 1991), and (iv) the toxic P effect (Loneragan et al. 1979; Marschner and Cakmak 1986; Webb and Loneragan 1988; Parker et al. 1992; Parker 1993). Soil factors that can contribute to the P/Zn interaction are few and have been attributed to P enhancement of the sorption of Zn to variable charge surfaces in the soil (Stanton and Burger 1967; Bolland et al. 1977).

The ratio of P/Zn has been suggested as a method of diagnosing Zn deficiency in crops
The Nitrate effect: Nitrate ($\text{NO}_3^-$) may affect Zn uptake by influencing the soil pH of the rhizosphere (rhizosphere pH). Zinc absorption is sensitive to changes in the rhizosphere pH and decreasing pH values increases Zn concentration in plants while increasing rhizosphere pH decreases Zn absorption (Wear 1956; Loneragan and Webb 1993). However, the effect of $\text{NO}_3^-$ on the uptake of Zn varies with plant species. For example, the rhizosphere pH of maize (\textit{Zea mays L.}) plants was 1.5 pH units higher than the bulk soil pH whereas the rhizosphere pH of chickpea (\textit{Cicer arietinum L.}) was 1.5 pH units lower even though $\text{NO}_3^-$ was supplied in both cases (Marschner and Romheld 1983).

Organic ligands effect: Organic ligands and chelating reagents (anions) have been demonstrated to increase Zn concentration in soil solution (Hodgson et al. 1966). However, the effect of organic ligands on Zn uptake by plants is inconsistent, as both increases and decreases in Zn uptake have been reported (Bell \textit{et al.} 1991). Possible factors that may control the effect of organic ligands on Zn uptake by plants include the residual charge on the complexed molecule, the specificity of the ligands for Zn complexation and the nutrient status of the plant (Laurie \textit{et al.} 1991). The availability of Zn complexes to plants may depend on the residual charge on the complex molecules. DeKock and Mitchell (1957) found those organic ligands with either one negative charge or none were more readily available for plant uptake than chelates with higher negative charge. Therefore, the uptake of Zn by mustard and tomatoes was higher when supplied with ammonia tri-acetic acid (ATA) rather than when supplied with ethylene diaminetetraacetic acid (EDTA) (DeKock and Mitchell 1957). Similarly, Zn uptake by maize plants was lower when supplied with FeEDTA than FeDTPA (Halvorson and Lindsay 1977) which could be due to the higher charge of ZnDTPA than ZnEDTA.

The uptake of metal-ligand complexes may be specific to the type of the ligand and to
Evidence for the role of phytosiderophores for Fe uptake in grasses is available, and it has been suggested that phytosiderophores in the rhizosphere can complex $\text{Zn}^{2+}$ and transport Zn into the root cell (Takagi et al. 1984; Kochian 1993).

**Total ions in solution:** The effect of single ions on Zn absorption has often been studied in simple solutions. However, in the more complex soil solution where a range of ions is present the effects of ions on the uptake of Zn by plants may be difficult to interpret. For example, in a solution of $\text{Ca(NO}_3\text{)}_2$ of Chaudhry and Loneragan (1972c), Zn absorption by wheat seedlings increased when pH increased from 3 to 7 whereas in a complete nutrient solution of Halvorson and Lindsay (1977) Zn uptake was reduced, when pH increased from 5.2 to 7.5. A chemical equilibrium program used to model the speciation of Zn in the complex soil solution suggested that the decrease in plant growth may have been due to the presence of synthetic organic ligands (EDTA or DTPA) that decreased the activity of $\text{Zn}^{2+}$ at high pH (Halvorson and Lindsay 1972; Halvorson and Lindsay 1977). In soil systems, the resultant effect of ions on Zn uptake would be a culmination of changes in (i) Zn adsorption on soil components, (ii) the formation of Zn complexes with organic ligands and (iii) the site of Zn absorption by roots.

**1.4.4 Movement of Zn**

Zinc is variable in its phloem mobility within plants compared to the fully mobile nutrients (N, K and P) and those that are phloem immobile (Ca, Mn) (Loneragan 1975; Loneragan et al. 1976; Longnecker and Robson 1993).

The first comprehensive study of distribution and redistribution of Zn was by Riceman and Jones (1958a, 1958b, 1958c, 1960a, 1960b) with clover and the study provided considerable insight into the movement of Zn in that plant species. Recently, Longnecker and Robson (1993) suggested that the amount of Zn supplied and the species of plant
affect the distribution and transport of Zn. This variability in phloem mobility could often explain the contradictory findings on Zn mobility within plants reported in the literature.

When plants have an adequate supply of Zn, Zn concentrations are usually higher in new growing (young) tissue than in mature vegetative tissue. That is, Zn is retranslocated to a greater extent at adequate supply compared to where Zn is low to deficient (Riceman and Jones 1958a; McGrath and Robson 1984; Isarangkura et al. 1978). Riceman and Jones (1958a) showed that the concentration of Zn in leaves declined as Zn accumulated in inflorescences, burrs and seed of clover. The movement of Zn from old leaves is delayed by Zn deficiency. In Zn deficient clover plants more of the total Zn in leaves was present in the blade than in the petiole (Riceman and Jones 1958b, c). A delay in senescence of old leaves (e.g. adequate to luxury N supply) can delay the movement of Zn from those leaves (Hill et al. 1979; Soliman et al. 1979).

The forms of Zn in either the xylem or phloem solutions are unknown. However, evidence from sap content and transport studies all suggest the complexation of Zn in the transport streams of plants (White et al. 1981a, b, c; Wanbite et al. 1981; McGrath and Robson 1984).

1.5 Diagnosis and prognosis of Zn deficiency

1.5.1 Introduction

Dry matter and grain yield of a crop are determined by the ability of the roots of plants to extract nutrients from the soil at absorption rates that are non-limiting for growth. Therefore, the availability of nutrients is often measured in terms of the quantity of nutrient absorbed by the plant (Beckwith 1963).

Plant analysis uses either selected plant parts (e.g. leaves, young growth), or whole shoots
of plants (Smith 1980). Plant analysis is generally used as a tool for diagnosing poor plant growth rather than a method of preventing a deficiency. This is in contrast to soil tests that generally predict rather than diagnose Zn deficiency. The lapse of time between sampling of the plants or plant parts, and the confirmation of a deficiency by plant analysis and then the delay until the deficiency can be ameliorated often results in yield losses since plant growth has been limited for this period of time (Smith 1980). It is for this reason that plant analysis has also been adapted for prognosis of deficiency (see Smith and Loneragan (1997)). Similarly, plant analysis does not allow for possible interactions between soil factors controlling the availability of nutrients to the plants and the metabolic responses of the plant involved in absorbing nutrients from the soil (Loneragan 1975). Bell (2000) suggested that temporary and or transient nutrient deficiencies present a particular difficulty for diagnosis or prediction of nutrient deficiency problems in plants. For example, in cool periods of the growing season when Zn uptake is limited, plant analysis at this time would suggest that plants are Zn deficient but as temperature increases the plants often recover without any grain yield decline (see section 1.6.8).

1.5.2 Symptoms of Zn deficiency

As Zn is essential for a range of enzymes and is associated with a range of other enzyme systems (section 1.1), deficiency of Zn disrupts many biochemical processes in the plant, which can be manifest in distinctive symptoms. Symptoms of Zn deficiency are often observed as interveinal chlorosis of leaves, production of small, often distorted leaves (little leaf), shortened internodes (rosetting) and death of the growing point (Viets et al. 1954b; Wallace 1966; Moraghan 1978; Bould et al. 1983; Reuter 1975; Marschner 1986). These symptoms occur in: citrus (Bar-Akiva et al. 1971); fruit trees (Bould et al. 1983); legumes (Riceman and Jones 1958a); flax (Loneragan 1951); and seedlings of P. radiata
Lesions and brown spots may develop on young leaves of lupin and there may be reductions in seed number, and weight of mature burrs of clover (Viets et al. 1954a; Riceman and Jones 1958c). In clover, root growth, nodule size and proliferation of nodules may be decreased by Zn deficiency (Reuter et al. 1982a). Red pigment may appear in stems and petioles of older leaves of peanuts grown under Zn deficiency (Bell et al. 1990). Zinc deficiency has been found to delay the pod maturity in navy bean (Phaseolus vulgaris L.) (Boawn et al. 1969; Wade and Bath 1985; Blaylock 1995).

In wheat the symptoms of Zn deficiency are usually observed early in young seedlings. The degree and extent of the symptoms depend on the severity of the deficiency of the soil. One symptom suggestive of mild Zn deficiency is a tramline pale strip each side of the mid-rib which extends and leaves collapsed in the middle as the deficiency worsens (Brennan 1986; Wurst et al. 1998). As the deficiency becomes more severe there is general paling of leaves and stunted plants are often observed to have “succulent” or “diesel soaked” looking leaves, showing necrotic areas about half way along the leaf (Brennan 1986).

The appearance of symptoms can vary with plant age, environmental conditions, severity and stage of the deficiency, as well as the supply of other nutrients. The symptoms may be slight or transient and either masked by other nutritional deficiencies or by unrelated factors such as disease and weather (Reuter 1975; Brennan et al. 1993). Probably the major failing of deficiency symptoms as a diagnostic tool for Zn is the fact that deficiencies are usually quite severe before symptoms appear on vegetative plant tissues. For example, there was about a 40 % reduction in yield of clover shoots before Zn deficiency symptoms were observed (Carroll and Loneragan 1968, 1969). Zinc deficiency symptoms may be confounded by disease or insect damage, weather conditions
or by other nutritional disorders and hence need to be considered with caution. For example, Hamilton et al. (1993) noted that early season symptoms of Zn deficiency did not result in a decrease in seed yield. Despite the limitations outlined, the symptoms of Zn deficiency for some crop species are so characteristic that positive identification is possible.

1.5.3 Diagnosis of Zn status by plant analysis

Yield reductions of 5 to 25 % are common with Zn deficiency (Brennan et al. 1993, Brennan 1992, 2000) but not accompanied by visual symptoms. Therefore, procedures for assessing the Zn status of plants by analysis (whole plant or tissue) are essential.

Diagnosis interprets the Zn status of the plant at the time of sampling (Melsted et al. 1969). Plant analysis for Zn needs to be related to specific growth stages of plant development. By measuring Zn concentrations in plant parts and relating the concentrations to dry matter yield, critical concentrations (usually defined as 90 % of maximum yield) can be defined for the plant species and for the particular time of sampling (growth stage) (Melsted et al. 1969; Ohki 1977; Ohki and Ulrich 1977).

The generalised relationship between nutrient concentration and yield is often described by the curve in Figure 1.1a. Yield increases rapidly with increasing nutrient concentration in the plant tissue, and then reaches a plateau when the nutrient is non-limiting for growth. As the nutrient concentration increases further plant growth declines, as the nutrient becomes toxic for growth.

A variation in the generalised relationship, known as the “C-shaped” or “Piper-Steenbjerg” curve has been reported for several nutrients, including Zn (Piper 1942; Steenbjerg 1951). The C-shaped curve (Figure 1.1b) may be due to: (i) relatively greater loss of dry matter than of Zn from older leaves with increased concentrations of Zn; (ii) a
higher requirement of Zn during early stages of growth together with delayed development due to severe Zn deficiency and (iii) under severe Zn deficiency the plant has no ability to grow but nutrient uptake continues (Hiatt and Massey 1958). The Piper-Steenbjerg relationship can confound the interpretation of results from plant analysis of whole shoots. For this reason, Andrew et al. (1981) were reluctant to quote critical concentrations of Zn in whole shoots of a range of tropical and subtropical legumes. Ulrich and Hills (1967) suggested that the problems associated with Piper-Steenbjerg curves for diagnosis could be minimised by plant sampling when symptoms first appear.

Figure 1.1 Relationships between yield and nutrient concentration in plant tissue. (a) Generalised relationship frequently found in plants as nutrient supply increases from deficient to toxic; (b) The C-shaped or Piper-Steenbjerg effect.

Both whole plant shoots (e.g. Viets et al. 1954a) and plant parts have been considered in diagnosis of Zn deficiency (see Reuter et al. 1997a; Brennan et al. 1993). Critical concentrations in whole shoots vary according to plant age, plant species, cultivar, and the environment (Ulrich 1952; Bates 1971). Leaves have been considered to be the most appropriate part of the plant to sample for nutrient status (Bates 1971) but the preferred age and location of leaves selected varies with the nutrient. Plant samples of the leaves and petioles of clover of varying age (composite sample of leaves) were considered unsuitable for assessing the Zn status of clover as the concentrations found in both Zn
deficient and Zn sufficient plants were similar (about 14 mg Zn/kg) (Riceman and Jones 1958c). Reuter et al. (1982a) found that Zn concentrations in individual leaves varied with Zn supply, plant age, leaf part and physiological age. Reuter et al. (1982a) showed about a 3 fold difference in Zn concentration between the leaves and stems of the same plants. The Zn supply also affected the distribution of dry weight among plant parts (Reuter et al. 1982a). Hence, Reuter et al. (1982a) concluded that it was necessary to use plant samples composed of a single plant part of the same physiological age and recommended the youngest open blade to diagnose Zn deficiency in clover.

Critical concentrations of Zn in youngest mature tissue have been successfully used for assessing the Zn status of an extremely wide range of plants, for example: apples (Watkins 1982), clover (Reuter et al. 1982a; Snowball and Robson 1983), pine trees (McGrath and Robson 1984), navy bean (Armour et al. 1989), peanuts (Bell et al. 1990), canola (Huang et al. 1995; Chapter 6) and pulse species (Snowball and Robson 1986; Armour et al. 1990; Chapter 6). Genc et al. (2002) showed that the critical Zn concentration in the youngest emerged leaf was a good indicator of the Zn status of barley plants, irrespective of the efficiency of Zn uptake by the genotypes.

Zinc concentration in the youngest open blades/leaves is stable at most stages of plant development (e.g. clover; Reuter et al. 1982a) reducing the risk of mis-diagnosis due to differences in sampling time. However, the Piper–Steenbjerg relationship between yield and Zn concentration has been reported for young tissue, such as sugar beet leaves (Rosell and Ulrich 1964) and apical growth of clover (Reuter et al. 1982a).

For tree crops, the Zn status is also determined by leaf analysis (Leece 1976; Bould et al. 1983). For perennial tree crops, recently mature leaves associated with an active growing phase of the tree were sampled (Leece 1976). However, Bould et al. (1983) suggested that for diagnosing Zn deficiency, young leaves instead of mature leaves be used for tree crops.
1.5.4 Biochemical assays

Biochemical tests, based on the activity of enzymes or metabolites that are specific for a particular nutrient, can be used to assess nutrient status in plant tissue. It has been suggested that biochemical tests provide a better estimate of the nutrient status of the plant particularly when tissues contain a large fraction of "physiologically inactive" nutrients (Leece 1976; Gibson and Leece 1981).

The activity of carbonic anhydrase has been used as an indicator of Zn deficiency in citrus (Day and Franklin 1946; Bar-Akiva and Lavon 1969; Bar-Akiva et al. 1971), rice, wheat and mustard (Dwivedi and Randhawa 1974), spinach (McMull and Bouma 1973) and maize (Gibson and Leece 1981). Zinc deficiency in citrus trees consistently lowered the activity of carbonic anhydrase to about 25 % of the activity in Zn-adequate trees (Bar-Akiva and Lavon 1969).

Bar-Akiva et al. (1971) suggested that the activity of carbonic anhydrase might be a useful indicator where the total Zn concentration in the tissue is likely to provide a poor indication of Zn deficiency. For example, where leaves contain high concentrations of Zn as a result of a foliar spray, a large proportion of the Zn may either be adsorbed to the surface of leaves or bound in other unavailable forms. In this case, the presence of a "physiologically inactive" pool of Zn in plant tissues may reduce the value of chemical analysis as a diagnostic procedure (Leece 1976, 1978; Gibson and Leece 1981). Hence, it has been suggested that the activity of carbonic anhydrase may provide a better indication of the Zn status of plants (Leece 1976; Gibson and Leece 1981). However, Dell and Wilson (1989) found that chemical analysis of plant parts was more sensitive than carbonic anhydrase assay for determining the Zn status of Eucalyptus maculata seedlings. In addition, other micronutrient deficiencies (Mn, Mo and Cu; see Bar-Akiva and Lavon 1969) have been found to depress the activity of carbonic anhydrase reducing the specificity of the test for
diagnosing Zn deficiency.

The activity of ribonuclease has been used to diagnose Zn deficiency in rice and maize (Dwivedi and Takkar 1974). An effect of low Zn supply on the activity of ribonuclease was observed before chemical analysis of plant tissue suggested Zn deficiency. Therefore, enzyme activity was more sensitive to Zn supply and possibly a better method to diagnose Zn deficiency.

The activity of aldolase enzyme has been used to diagnose Zn deficiency in onion (O’Sullivan 1970). However, Bar-Akiva et al. (1971) failed to establish a specific Zn requirement for the aldolase enzyme in lemon leaves.

Biochemical assays have not gained wide acceptance for the diagnosis of nutrient deficiencies, probably because of both their complexity (e.g. sampling, preserving tissue) and their lack of specificity as discussed by Robson (1981). The development of enzyme tests for Zn deficiencies for a range of species would require an immense amount of enzyme calibration work.

1.5.5 Nutrient balances and ratios

A range of nutrient ratios, P/Zn (Millikan 1963; Schropp and Marschner 1977), Fe/Zn (Rashid et al. 1976; Nambiar and Motiramani 1981), and Mn/Zn (Nair and Probhat 1977) have been suggested as tools for diagnosing Zn deficiency for a range of crops. However, other researchers have found no relationship between P/Zn ratios in corn (Giordano and Mortvedt 1969) or clover (Reuter 1980) and Zn deficiency. As Zn maintains root membrane integrity (Welch et al. 1982), Zn deficient plants accumulate excess ions. Such accumulations of ions in Zn-deficient plants may render nutrient ratios unreliable for diagnosis of Zn deficiency. In addition, unless a nutrient affects the utilisation of Zn within the plant it is unlikely that the specific nutrient/Zn ratio is of any significance in diagnosing
Zn-deficiency. The ratios appear to vary widely with species, genotype and possibly with environmental conditions.

Another approach for nutrient deficiency diagnosis is based on the notion of optimal balance ratios adopted by the Diagnostic and Recommendation Integrated System (DRIS). DRIS interpretation is based on calculated elemental ratio indices compared to established norms (Beaufils 1973). For Zn, DRIS is frequently misleading, as the Zn database is small. Moreover, norms are not independent of time of sampling or location. For example, DRIS failed to detect 8 of 11 Zn deficiencies of soybean (Hallmark et al. 1989).

1.5.6 Measurement of plant available Zn by soil analysis

Introduction: The objectives of a soil test are to (i) group soils into classes for fertiliser recommendations, (ii) predict the probability of a response to an application of the nutrient and (iii) evaluate soil productivity (Hibbard 1940; Fitts and Nelson 1956). A good soil test should meet three criteria; (i) the reagent should extract all or a proportionate part of the available form or forms of a nutrient from soils, (ii) the amount of the nutrient extracted should be measured with accuracy and minimal time delay (speed) and, (iii) the amount extracted should be correlated with the growth and response of each crop to that nutrient under various conditions (Bray 1948; Cope and Evans 1985; Sims and Johnson 1991).

Soil test for Zn: Soil testing to measure plant available Zn has been reviewed by Bauer (1971), Brown et al. (1971), Cox and Kamprath (1972), Viets and Lindsay (1977), Cox (1987), Sims and Johnson (1991), and Brennan et al. (1993). In addition, studies have evaluated chemical methods of extracting Zn from soils for a range of plant species (Hibbard 1940; Wear and Sommer 1948; Tucker and Kurtz 1955; Wear and Evans 1968; Alley et al. 1972; Evans et al. 1974; Tiwari and Kumar 1974; Sedberry et al. 1979; Shang and Bates 1987; Sims and Johnson 1991; Rodriguez et al. 1999). Similar studies have evaluated various soil factors affecting the extraction of Zn by various chemical reagents.
from soils (Singh et al. 1983b; Sims and Johnson 1991). Micronutrient soil tests are used to predict a deficiency but are rarely expected to predict the quantity of Zn required for maximum yield. However, since the amount of micronutrient fertiliser required is usually within a narrow range (e.g. 4 to 10 kg/ha for Zn) (Lindsay 1972a) the main purpose of the Zn soil test is to predict where deficiency is likely.

Zinc availability in soils is generally measured by the use of chemical reagents (extractants) that remove a fraction of the total soil Zn. Some extractants are used for the specific determination of Zn, while other extractants are used for simultaneous extraction of a number of plant essential nutrients (multi-element extractants eg Mehlich-3; e.g. Junus and Cox 1987; sodium bicarbonate-DTPA, Rodiguez et al. 1999). Another approach to Zn soil testing is to extract the various pools of soil Zn by chemical fractionation techniques in an attempt to provide some qualitative evidence of the association of Zn (and other micronutrients) with various soil fractions and their potential availability to plants (Lopez and Graham 1972, 1973; Harrison 1981). However, fractionation is not without problems, and the advantages and limitations of sequential chemical fractionation techniques arising from the choice of extractants, procedures, and methodology have been extensively reviewed (Beckett 1989).

The choice of a particular extractant for soil Zn tests is often on the basis of correlation between the amounts of Zn extracted and either plant yield or Zn uptake by plants. The correlation obtained for various soil test Zn-extractants is influenced by the soil/solution ratio, extraction time and temperature, soil properties (clay, pH), and chemical form of Zn in the soil (Leggett and Argyle 1983; Haddad and Weir 1985; Sajwan and Lindsay 1988; Armour et al. 1989; Brennan et al. 1993).

Bauer (1971) has listed 25 extractants that have been used to measure soil available Zn for plant growth and plant uptake of Zn. The extractants may be divided into 3 categories:
water and neutral salts; weak and strong acids; and chelating agents (Viets and Lindsay 1977). Total Zn is rarely used to predict plant available Zn as there is a poor of correlation between it and plant growth or uptake. The lack of correlation is probably due to the low proportion of the total soil-Zn taken up by the plant. For example, plant content of Zn was about 0.07 % of the total Zn in the soil (Viets 1962, 1966; Viets and Lindsay 1977).

Examples of early extraction procedures included dithizone in carbon tetra chloride (Shaw and Dean 1952); 0.1M HCl (Viets et al. 1954b; Nelson et al. 1959; Viets and Lindsay 1977; Singh and Shukla 1985); 2N MgCl₂ (Stewart and Berger 1965); 0.1M NaNO₃ (Hani and Gupta 1985); 0.05M CaCl₂ (Sauerbeck and Styperck 1985); 0.5M HNO₃ (Cottenie et al. 1982); NH₄OAc (Jensen and Lamm 1961) and Mg(NO₃)₂ (Shuman 1985b). The FAO has proposed a reference method using 0.5N NH₄ acetate+0.02M EDTA (pH 4.65) as the extracting solution for soil test Zn (Sillanpää 1982). Chelating extractants usually include EDTA and DTPA for example: 0.05M DTPA + 0.01M CaCl₂+0.1M TEA pH 7.30 (Lindsay and Norvell 1969); 1M NH₄HCO₃+0.005M DTPA (pH 7.6) (Soltanpour and Schwab 1977); (NH₄)₂CO₃+EDTA (Trierweiler and Lindsay 1969); 0.5N Na acetate+DTPA pH 4.8 (Wolf 1982). The latter reagents of chelates have generally superseded the earlier extraction procedures involving the water and neutral salts; and weak and strong acids.

Chelating agents react with cations in solution to form soluble complexes. As the activity of free metal ions in solution is decreased labile solid phases can then release Zn²⁺ into the soil solution. The amount of chelated metal in solution after extraction is considered to be a function of both the initial activity of the ions in solution (intensity factor, I) and the rate of replenishment from labile sources (quantity factor, Q) and thus to simulate the uptake of Zn by plant roots (Dhillon et al. 1975; Viets and Lindsay 1977).

Although EDTA and DTPA are commonly used chelating agents there has been considerable variation in the extraction methodology described for both extractants
(Brennan et al. 1993). For example, EDTA and sodium EDTA concentrations have ranged from 0.005 to 0.05 M, pH values from 7 to 8.6, with and without the addition of salts such as (NH$_4$)$_2$CO$_3$ and Ca(NO$_3$)$_2$ while soil/solution ratios have ranged from 1:2 to 1:10 and 0.5 to 1 hour extraction times have been used (Viro 1955; Trierweiler and Lindsay 1969; Brown et al. 1971; Dolar and Keeney 1971; Fujii and Corey 1986). For DTPA, similar variations in extraction procedures and methods have been reported (Dolar and Keeney 1971; Soltanpour and Schwab 1977). Differences in the shaking speed, the intensity of shaking and sample preparation procedures also affect the results of the various extraction methods (Soltanpour et al. 1976; Leggett and Argle 1983; Haynes and Swift 1991). Therefore standardisation of methods for Zn soil tests are required before data can be interpreted or compared across different studies.

However, many researchers have found poor correlation between the soil test Zn extractant and either the yield of plant shoots or Zn content in plant shoots (see review Brennan et al. 1993). Many of these researchers have included other soil properties in the relationship to improve the soil test for Zn. For example, Haq and Miller (1972) found that Zn uptake of corn grown on a range of soils was poorly correlated with EDTA- and DTPA-extractable Zn, however, including soil pH greatly improved the soil test for EDTA (the coefficient of determination, $R^2 = 0.75$) and for DTPA ($R^2 = 0.76$). Similarly, Pal et al. (1989) used the soil properties of texture and electrical conductivity to improve the ability of the DTPA-Zn soil test to predict Zn deficiency of maize grown in 26 soils of India. Therefore, the soil properties of soil pH, organic carbon (%), clay (%) and CaCO$_3$ (%) have been included in a multiple regression in an attempt to improve a soil test for Zn (see Table 1.3, Srivastava et al. 2000; McLaughlin et al. 2000).
Table 1.3. Some extractants and soil properties used to improve soil tests for Zn for a range of crops.

<table>
<thead>
<tr>
<th>Crop</th>
<th>Crop parameter</th>
<th>Soil test extractant</th>
<th>R²</th>
<th>Soil properties includeda</th>
<th>R²</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oats</td>
<td>uptake</td>
<td>0.1 M HCl</td>
<td>0.31</td>
<td>pH, OC, silt, Clay</td>
<td>0.70</td>
<td>Osiname et al. 1973</td>
</tr>
<tr>
<td>Oats</td>
<td>uptake</td>
<td>EDTA</td>
<td>0.33</td>
<td>OC</td>
<td>0.70</td>
<td>Osiname et al. 1973</td>
</tr>
<tr>
<td>Maize</td>
<td>concentration</td>
<td>0.1 M HCl</td>
<td>0.46</td>
<td>Bray-P, pH</td>
<td>0.67</td>
<td>Peaslee 1980</td>
</tr>
<tr>
<td>Maize</td>
<td>relative yield</td>
<td>0.1 M HCl</td>
<td>0.38</td>
<td>CEC, EC, P, BD, OC</td>
<td>0.87</td>
<td>Singh &amp; Takkar 1981</td>
</tr>
<tr>
<td>Maize</td>
<td>concentration</td>
<td>DTPA</td>
<td>0.32</td>
<td>CEC, EC, P, BD, OC</td>
<td>0.87</td>
<td>Singh &amp; Takkar 1981</td>
</tr>
<tr>
<td>Sorghum</td>
<td>uptake</td>
<td>MgCl₂</td>
<td>0.15</td>
<td>pH, OC, silt, clay</td>
<td>0.36</td>
<td>Chude &amp; Gabriel 1984</td>
</tr>
</tbody>
</table>

aOC is organic carbon; P is P extracted by the Bray method; CEC is cation exchange capacity; BD is the soil bulk density; EC is the electrical conductivity.

An alternative approach is to soil test for the intensity fraction of the soil Zn rather than those associated with quantity of Zn in soil (Nair 1984). Soil solutions can be extracted from soils at field capacity by centrifugation (Gillman 1976; Aitken and Outhwaite 1987) or by displacement methods (Sanders 1982). Lower detection limits of analytical instruments with stringent contamination controls has allowed the measurement of Zn in the soil solution although the routine analysis of soil solutions for Zn is rarely done at present.

McGrath et al. (1985) found that Zn concentration and uptake by clover shoots grown on four soil types was better correlated (significantly higher correlation coefficient (r)) with Zn concentrations in the soil solution than with Zn extracted by DTPA except for the calcareous clay soil. Tiller et al. (1972) has shown that intensity measurements were the better predictors of Zn concentration and Zn uptake in shoots of clover and wheat grown on 25 diverse Australian soils. Tiller et al. (1972) found that 1 mol/L MgCl₂, 0.05 mol/L Ca(NO₃)₂, 0.05 mol/L CaCl₂ extractions of Zn concentration were all significantly correlated with Zn uptake and concentration of plant shoots. However, the CaCl₂ extraction of Zn had the highest correlation coefficient suggesting its suitability for measuring intensity of Zn. An important conclusion of Tiller et al. (1972) was that extractants of Zn quantity did not predict plant Zn uptake or concentration unless restricted to soils with similar soil properties. However, the extractants of Tiller et al. (1972) had high ionic strength and
hence could be considered to extract more than just soil solution Zn. The ionic strengths of the solutions used in Tiller et al. (1972) study were 3M for MgCl₂, 0.15M for Ca(NO₃)₂ and 0.15M for CaCl₂. The ionic strength of the solutions used in Tiller et al. (1972) contrast with an average ionic strength of 6 soils from Queensland of about 0.005M (Gillman 1981; Gillman and Bell 1978) and about 0.0048M for 20 soils of West Australian (Dolling and Ritchie 1985). Thus, the 'intensity' measurements of Tiller et al. (1972) could be extracting considerably more Zn than available in the soil solution of these soils.

Multi-nutrient extractants, those soil test extractants used for both macro- and micro-nutrients in the one chemical extraction procedure, have been previously examined for use as a soil test for Zn (Junus and Cox 1987). Several authors have recently re-examined these reagents for soil testing for Zn (for example, Rodriguez et al. 1999; Abreu et al. 2002; Cancela et al. 2002). The multi-nutrient soil test reagents and procedures have the same problems as single extractants as discussed above. The multi-element soil tests have had varying degrees of success. A multi-nutrient soil test for Zn has not been calibrated for any crop species or for the range of soil types found in WA.

1.6 Plant availability of Zn in soils

The term 'availability' has a range of meanings but is commonly used to describe the ability of plants to utilise nutrients in the soil (Tiller 1983).

1.6.1 Factors affecting the availability of Zn in soils to plants

Factors affecting the solubility of Zn in soils and availability to plants has been extensively reviewed by several authors (Hodgson 1963; Mitchell 1964; Sillanpää 1972; Lucas and Knezek 1972; Knezek and Ellis 1980; Pulford 1986; Kiekens 1990; Marschner 1993; Catlett et al. 2002). The factors affecting Zn availability to plants are total Zn content, soil pH,
organic carbon, clay content and Fe and Al oxides (adsorption sites), microbial activity, soil moisture regimes, root distribution and rhizosphere effects. These are reviewed in the following sections.

1.6.2 Zn concentration of soils

Soils with a low total Zn concentration are often Zn deficient for crop production. Since quartz contains negligible Zn, sandy soils are inherently low in total Zn concentration and frequently deficient in available Zn. Zinc deficiencies of plants grown on acid soils are generally associated with low total soil Zn concentration (Lucas and Davis 1961). These cases of Zn deficiency are associated with an absolute deficiency of Zn.

Total soil Zn concentrations in calcareous soils are often similar to or higher than those of non-calcareous soils but Zn deficiency is frequently reported for calcareous soils (Thorne 1957; Ravikovitch et al. 1968; Yoshida and Tanaka 1969). Therefore, although total soil Zn concentration in calcareous soils is high, much is unavailable for uptake by roots of plants. That is, the Zn is “unavailable” due to effects of soil pH (see 1.6.5 for soil pH effect on Zn availability).

Distribution of total Zn with depth down the soil profile has been reported to be uniform, while concentrations of extractable Zn measured by various chemical reagents decrease markedly with depth down the soil profile (Tiller 1963; Follet and Lindsay 1970; Lindsay 1972a). There is no data on the distribution of total or extractable Zn in soil profiles from WA.

1.6.3 Effect of restricted root zones and exposed subsurface soil on Zn uptake

Plants grown in soils where root growth is restricted often show Zn deficiency. A natural hardpan (zone of high soil bulk density), or traffic pan, a high water table or very acid subsoil (Lucas and Knezek 1972) can cause poor root proliferation through the soil profile.
and thus limit access to soil Zn for plant uptake. Similarly, peat and muck soils (Histosols) are often Zn deficient, because the plant roots are present in the surface layer while the mineral forms of Zn available for plant uptake are in the deeper soil profile (Lucas and Knezek 1972). Similarly, Zn deficiency in crops (Grunes et al. 1961) and pastures (Kleinig and Loveday 1962) has been caused by removing topsoil (‘scalping’) during land levelling for irrigation. The exposed sub-surface horizon is often less enriched with Zn and sometimes has a higher pH and carbonate content (so Zn in soil is less available) than the removed topsoil. Severe soil erosion could produce similar effects.

1.6.4 Root interception

The interception of nutrients by roots is an important mechanism for the uptake of soil immobile nutrients, such as Zn (Wilkinson 1972; Mortvedt and Gilkes 1993). The frequency of root interception increases with granular fertilisers having lower concentration of Zn as more granules are required to provide the same level of Zn application (Mortvedt and Gilkes 1993). For example, to supply 6 mg Zn to 3 kg soil, the number of granules needed was 8 when the concentration of Zn in the granule was 8 %. However if the Zn concentration in the granule was 0.5 % the number of granules increased to 64 (from Giordano and Mortvedt 1966). In the work of Giordano and Mortvedt (1966) the yield of corn grown on a sandy clay loam (non-calcareous, soil pH 7.3) was reduced by 53 % when the number of granules was decreased from 64 to 8. Giordano and Mortvedt (1966) showed about a 68 % decrease in yield when the Zn concentration in ammonium pyrophosphate granules increased from 0.5 to 8 % (that is, as the number of granules decreased). Similarly, Hoeft and Walsh (1971) using Zn frits incorporated in single superphosphate (superphosphate) showed a decrease in yield as the number of Zn containing particles decreased. Although the effects of Cu distribution and granular fertiliser size on cereal grain yield has been reported in WA for Cu-containing fertilisers (Gartrell 1981), there appears to
be no such data for Zn fertilisers.

1.6.5 Effect of soil pH on the availability of Zn

The pH of soil is an important factor affecting the availability of Zn. At high soil pH, Zn is more strongly absorbed onto the surface of silicate clays and oxides (Lindsay 1978, 1981; Jeffery and Uren 1983; Harter 1983; Jahiruddin et al. 1985, 1986) and hence the availability of Zn to plants is diminished. An increase in soil pH decreases Zn availability for plant uptake (Shaw and Dean 1952, Seatz et al. 1959; Seatz 1960) and liming (addition of calcium carbonate) often leads to Zn deficiency in plants (Lopez 1980). A greater incidence of Zn deficiency is also reported in calcareous soils as Zn in the soil is in unavailable forms for plant uptake (Thorne 1957; Ravikovitch et al. 1968; Navrot and Ravikovitch 1969; Gupta et al. 1987). The acidification (lowering of soil pH) of soils increased the concentration of Zn available to plants (Boawen et al. 1960). Over the soil pH range of 5.5 to 7.0, the Zn concentration in plants may decrease by 3 to 4 times for each one unit increase in soil pH (Wear 1956; Barber 1984; Sims 1986; Moraghan and Mascagni 1991). The addition of lime to acid soils decreased the concentration and content of Zn in plants (Grove and Sumner 1985; Parker and Walker 1986) and increased the risk of inducing Zn deficiency in plants grown on weathered tropical soils (Duguma et al. 1988). Conversely, placing Zn with acid-forming fertilisers (Mortvedt and Kelsoe 1988) increased the availability of applied Zn to plants.

Alkaline soils are often quoted as requiring higher levels of Zn to be applied for maximum plant growth than acid soils (Lindsay 1972a; Clark and Graham 1968; Saeed and Fox 1977). In alkaline, calcareous soils there may be precipitation of Zn(OH)$_2$, ZnCO$_3$ or calcium zincate compounds of lower availability for plant uptake (Rogers and Wu 1948; Clark and Graham 1968; Saeed and Fox 1977) or the adsorption of Zn by carbonates (Udo et al. 1970). In addition high solution Ca inhibits Zn uptake and this may also be involved in the apparent
reduced availability of Zn in alkaline and limed soils (Chaudhry and Loneragan 1972a, c).

**Effect of N sources on soil pH:** Ammonium based fertilisers may increase Zn uptake by decreasing soil pH. The availability of native and applied Zn to plants was markedly increased where ammonium sulfate reduced the soil pH from 7.2 to 5.0 but decreased when sodium nitrate was applied (soil pH 7.3) (Viets et al. 1957). Similarly, Giordano et al. (1966) found that the yield and Zn content of corn plants were higher when ammonium sulfate was applied than when anhydrous ammonia or urea was applied. For cereals and canola (commonly called rape or oil-seed rape) it has been shown that the acid-forming N sources (such as ammonium sulfate), resulted in the Zn content of young plants being about 30 % greater than in plants that were supplied with ammonium nitrate (Schnug and Finck 1982).

The addition of nitrate and ammonium may change the soil pH of the rhizosphere of plant roots (rhizosphere pH) to a greater extent than to the soil pH of the entire soil. As Zn is immobile in soils, the rhizosphere pH may have a larger effect than the entire soil pH on the availability of Zn to plants (Wilkinson et al. 1968). Hence, the addition of nitrate and ammonium probably influences the availability of Zn to plants by changing the rhizosphere pH (Marschner 1991, 1993). Increase in rhizosphere pH for plants supplied with nitrate-N decreases both the concentration and mobility of Zn (Marschner 1991). Marschner (1993) postulated that the increase in rhizosphere pH for plants supplied with nitrate is prominent in plants species which reduce nitrate predominately in the roots, as for many perennial species such as fruit trees. However, in dicot species that are Zn deficient a decrease in rhizosphere pH occurs even when nitrate N is supplied. The acidification of the rhizosphere is due to an increase in the cation/anion uptake ratio (Cakmak and Marschner 1990). Cakmak and Marschner (1988a) showed that the permeability of root cells is increased with an efflux of sugars, amino acids and phenolic compounds. However, only the root exudates of
graminaceous species are effective at mobilizing Zn from Zn loaded resin or from calcareous soil (Marschner et al. 1990). Marschner and Romheld (1983) showed that the rhizosphere pH of maize plants was about two pH units lower than the soil pH of the bulk soil when NH$_4^+$ was applied. Such a decrease in rhizosphere pH would considerably increase the availability of Zn in the soil for plant uptake (Nye 1986). Besides the increase in solubility and mobility of Zn through root-induced changes, the presence of higher activity of non-infecting rhizosphere microorganisms may indirectly affect the availability of Zn, for example chelators of Zn produced in the rhizosphere (Merckx et al. 1986; Linehan et al. 1989).

1.6.6 Effect of soil organic matter on Zn availability

Zinc deficiency of crops has been reported for plants growing on peats (high organic matter content) and on areas where large amounts of organic matter had been added (e.g. hay stacks, straw and stubble areas). Zinc may be bound to organic compounds that are unavailable for plant uptake resulting in Zn being less available for uptake by roots of plants (Himes and Barber 1957; Mortensen 1963; Lindsay 1972b). However, as discussed in section 1.3.5 and in section 1.6.7 the adsorption of Zn by organic ligands and organic components of organic matter can have both positive and negative effects on Zn availability.

1.6.7 Effect of plant root exudates and soil micro-organisms on Zn availability

Plant root exudates and microbial by-products that form soluble organic complexes with Zn may facilitate the absorption of Zn by roots of plants (Merckx et al. 1986; Marschner et al. 1987; Treeby et al. 1989; Marschner 1991).

Zinc concentration in the shoots of rice plants (60 days after transplanting) grown in the glasshouse on a silty clay loam (pH 8.3) with added ground rice straw increased by about 50 % compared to the Zn concentration of rice plants grown without a straw amendment (Singh
et al. 1985). This may indicate that the production of organic acids during decomposition of the straw by microorganisms has increased the availability of Zn. The addition of straw may also lower the redox potential of the soil and available Zn to rice plants may be decreased through this mechanism also. In contrast, the decrease (about 36 %) in ammonium acetate extractable Zn in the presence of starch added to a lateritic soil may be due to microbial immobilisation of the Zn (De Remer and Smith 1964; Mandal and Mandal 1987).

An important effect of VA mycorrhizae is to improve the supply of nutrients of low mobility in the soil (eg P, Zn, and Cu) (Lambert et al. 1979; Bell et al. 1989; Thompson 1990; Kothari et al. 1991). The external hyphae of VA mycorrhizae absorb and translocate Zn to the host plant (Bell et al. 1989; Ryan and Angus 2003). The VA mycorrhizae have been shown to increase Zn uptake in soils low in extractable Zn, for example, peaches (Gilmore 1971), citrus (Menge et al. 1982), apple (Runjin 1989), corn (Faber et al. 1990) wheat (Swamvinathan and Verma 1979; Ryan et al. 2002; Ryan and Angus 2003) and barley (Jakobsen 1983). The effectiveness of VA mycorrhizae to absorb Zn seems to be maintained when plants are grown at high soil Zn supply (Dueck et al. 1986; Schuepp et al. 1987).

Roots of graminaceous species, including wheat, release phytosiderophores into the soil under Zn deficiency (Takagi et al. 1984; Kawai et al. 1988; Romheld and Marschner 1990; Marschner 1991; Cakmak et al. 1994, 1996a; Walter et al. 1994; Hansen and Jolley 1995; Hansen et al. 1995, 1996; Rengel and Graham 1995a, b; Rengel et al. 1998). Durum wheat genotypes which are sensitive to Zn deficiency (Cakmak et al. 1994, 1996b, c, d; Walter et al. 1994; Rengel 1999) exude a relatively small amount of phytosiderophores under Zn deficiency (Cakmak et al. 1994, 1996b; Rengel et al. 1998). By contrast, wheat genotypes tolerant to Zn deficiency have increased exudation of phytosiderophores (e.g. 2-
deoxymugineic acid (DMA)) under Zn deficiency, although Erenoglu et al. (1996) suggested that the release of phytosiderophores is not well correlated with Zn efficiency in wheat genotypes. The exudation of phytosiderophores by wheat genotypes and the reduced Fe transport to shoots has been suggested as a physiological mechanism for the genotypic tolerance to Zn deficiency (Rengel and Graham 1995a; Walter et al. 1994). The reduced Fe transport from the root to shoot under Zn deficiency would result in physiological Fe deficiency, which in leaves has been suggested as a trigger for the increased exudation of phytosiderophores. However, only a single study (Walter et al. 1994) that involved a wheat cultivar tolerant and a durum wheat cultivar sensitive to Zn deficiency has tested the relationship between Fe deficiency and differential tolerance to Zn deficiency. Hence, the role of phytosiderophores in Zn uptake by wheat and other graminaceous species requires further elucidation.

1.6.8 Effect of plant species and varieties on Zn concentration in plants.

Plant species differ in their Zn requirement (Ponnamperuma 1972; Shukla and Raj 1974; Clark 1976; Tiwari and Pathak 1982; Moraghan 1984; Graham and Rengel 1993; Cakmak et al. 1996d; Rengel 2001). Legumes (lupins, clover) generally have higher Zn concentrations in plant shoots than cereals and grasses when grown in the same soil. Gladstones and Loneragan (1967) suggested that species differ in their "feeding power" for Zn. Within the cereals, the susceptibility to Zn deficiency was found to decline in the order durum wheat > oat > bread wheat > barley > triticale > rye (Cakmak et al. 1996c, 1997, 1998; Erenoglu et al. 1999; discussed above in phytosiderophores (1.6.7)).

Genotypes of a range of species grown in soil with low extractable Zn show differences in the incidence of, and depression of growth due to, Zn deficiency (Rengel 2001). Genotypes of wheat (Graham et al. 1992; Cakmak et al. 1994, 1996b; Rengel and Graham 1995a), rapeseed (canola) (Grewal et al. 1997a, b), chickpeas (Khan et al. 1998, 2000) and durum
wheat (Cakmak et al. 2001) differ in their tolerance to Zn deficiency. Genotypes of the pasture species of lucerne (alfalfa; *Medicago sativa* L.) (Grewal and Williams 1999) and annual medics (*Medicago* spp.) (Streeter et al. 2001) also have been found to differ in susceptibility to Zn deficiency.

Genc et al. (1998, 2002) showed that the growth increase to Zn fertiliser and deficiency symptoms were more severe on an inefficient genotype compared to a Zn efficient genotype of barley. In Zn efficient navy bean Zn concentrations in plant tissue were similar to (Polson and Adams 1970; Edwards and Mohamed 1973) or greater than (Ambler and Brown 1969; Mugwira and Knezek 1971, Jolley and Brown 1991) those in an inefficient navy bean cultivar. Moraghan and Grafton (1999) found that Zn efficient genotypes of navy bean had >25 % higher concentrations of Zn in the seed than the inefficient genotypes when grown on the same soil type. It was suggested that selection of genotypes with higher seed-Zn concentration could be made for Zn efficiency (agronomically) and as a better source of seed Zn for human nutrition (Moraghan and Grafton 1999). The relationship between Zn-efficiency and high seed-Zn concentration does not occur in all crops. For example, a Zn-efficient wheat cultivar had a lower grain Zn concentration than an inefficient cultivar (Graham et al. 1992; Rengel and Graham 1995a).

The mechanisms responsible for the differences in Zn efficiency for plants are not fully understood. However, efficient species and genotypes are characterised by greater Zn acquisition from the soil by modifications to root morphology in the form of longer and thinner roots (Dong et al. 1995). Similarly, for a range of plant species under Zn deficiency the root:shoot ratio has been found to increase (Cumbus 1985; Loneragan et al. 1987; Khan et al. 1998). Differences in efficiency among plant species are often related to inherent differences in soil rhizosphere pH, root exudation, or colonisation by VA mycorrhizae (Rengel 1997, 1999). Several authors have characterised the genes and loci for Zn
efficiency in crop species and genotypes of wheat and rye (Schlegel *et al.* 1998), of rice (Thongbai *et al.* 1999), common bean (Singh and Westermann 2002) and barley (Genc *et al.* 1998, 2002).

1.6.9 Effect of soil temperature and light intensity on Zn availability

Zinc deficiency symptoms can occur during the cool season and then disappear as the temperature rises (Ozanne 1955b; Martin *et al.* 1965, Rudgers *et al.* 1970; Giordano and Mortvedt 1978; Brennan *et al.* 1993). Zinc uptake by potato plants grown on a sandy loam increased with increasing soil temperature (Martin *et al.* 1965). Bauer and Lindsay (1965) grew corn in the glasshouse on a loam soil that was previously incubated for one week at a range of different temperatures. The uptake of Zn by plants that were grown in soil that was incubated at 43°C was significantly higher than for plants grown in soil that was incubated at lower temperatures suggesting the involvement of reduced rate of mineralization of Zn by soil microbial activity in the elevated pool of plant available Zn.

For soils low in extractable Zn, low soil temperature often enhances the incidence and severity of Zn deficiency symptoms (Rudgers *et al.* 1970; Moraghan and Mascagni 1991). Schwartz *et al.* (1987) showed that Zn uptake by shoots of barley grown in solution at 20°C was 83 % higher compared to the Zn content of plants grown with a root temperature of 10°C. Roots of plants grown at a root zone temperature of 10°C developed fewer secondary roots, and were thicker and shorter in length compared with roots grown at the higher temperature. It was suggested that colder root temperatures reduce nutrient absorption and/or reduced translocation rates so that the shoots were deprived of adequate Zn supply (Schwartz *et al.* 1987). Similarly, in controlled experiments, temperature less than 16°C during growth of the plants were associated with a decrease in Zn content in shoots of maize (Ellis *et al.* 1964), linseed (Moraghan 1980), and tomato (Fawusi and Ormrod 1975).
Trumble and Ferres (1946) and Ozanne (1955b) suggested that short daylength and low temperatures increased the severity of Zn deficiency of pasture legumes under Australian winter conditions. Ozanne (1955b) found that reduced light intensities increased the severity of Zn deficiency and reduced the mean yield of shoots of clover grown in the glasshouse on a grey sand (pH 5.2) for 42 days. Millikan (1953) found that the uptake of Zn was reduced by about 53 % for clover and 65 % for lucerne plants grown under reduced light conditions compared to plants grown under full light conditions. However, the relative effects of both the low temperature and short daylength on the severity of Zn deficiency could not be deduced from this field work.

Recently, high light intensity and long-day length have been shown to be a major factor in the development of Zn deficiency symptoms (Edwards and Kamprath 1974; Marschner and Cakmak 1989). The effects of light and long-day length could be mediated through the roles of Zn in enzymes of photosynthesis (e.g. superoxidase dimutase and carbonic anhydrase), in protein synthesis and in reactive oxygen species (Marschner 1993).

1.6.10 Effect of soil moisture on Zn uptake

Under waterlogged conditions (anaerobic), the chemistry of Zn in the soil is altered compared to well-drained soils (aerobic). For example, when a soil is submerged (anaerobic), the concentration of water-soluble Zn decreases compared to well-drained (aerobic) soils (Ng and Bloomfield 1962; Ponnamperuma 1972; Mikkelsen and Shiou 1977). Similarly, Amer et al. (1980) showed that the water-soluble Zn concentration of a submerged alkaline clay soil (pH 8.0; 4.1 % CaCO₃) decreased by about 82 % after three weeks. The decline in the solubility of Zn and reduced uptake of Zn in poorly drained soils is attributed to the co-precipitation of Zn with soluble Al and Fe in the soil (Takkar and Sidhu 1979; Singh and Abrol 1986; Sajwan and Lindsay 1986). Precipitation of ZnS has also been suggested to reduce the availability of Zn to rice under flooded conditions.
Ponnamperuma (1972). Sajwan and Lindsay (1986) questioned the formation of ZnS and concluded that the reduced availability of Zn in flooded rice soils was due to higher levels of Fe$^{2+}$ and Mn$^{2+}$ suppressing the uptake of Zn and to the precipitation of ZnFe$_2$O$_4$. Moreover, plant factors in water-logged soil conditions such as impaired root respiration and metabolic processes may restrict Zn uptake especially in species that lack adaptation to anaerobic soils. However recently Kirk and Bajita (1995) concluded for rice, a species adapted to anaerobic soils, that Fe oxidation resulted in acidification of the rhizosphere that released Zn from highly insoluble fractions. Zinc released from these highly insoluble forms was re-sorbed on Fe(OH)$_3$ and organic matter in the soil of the rhizosphere in forms that were available for uptake by roots (Kirk and Bajita 1995).

Conversely, from lysimeter studies, it was found that improved soil drainage increases the uptake of Zn by plants. The Zn concentration in the ear-leaf of corn increased as the water level in the soil dropped from 15 cm, and from 30 cm to no water table; the increases being 72, 82 and 92 mg/kg, respectively (Lal and Taylor 1970). However, it is not clear how much the increase was due to greater root exploration and how much to the specific effects of waterlogging.

Generally, as the soil water content decreases the diffusion coefficient of Zn ions in soil would be expected to decline (Oliver and Barber 1966; Warncke and Barber 1972; Barber 1984; Sharma and Deb 1990). In drying topsoil, Nambiar (1975, 1976a, b, 1977a, b) found that oat and ryegrass plants were able to absorb some Zn for growth. Contrary to expectation, uptake of Zn occurred in roots growing through a layer of soil drier than wilting point provided that the roots had access to water in the subsoil (Nambiar 1976a, b). In dry soil, roots release more mucilage in response to the mechanical impedance, and this may aid Zn transport at the root–soil surface (Nambiar 1976b).
1.7 Effect of farming practices on Zn status

1.7.1 Fertiliser practices

Four strategies normally used to prevent Zn deficiency are: (i) soil applications; (ii) foliar applications; (iii) coating Zn fertilizer on seeds (seed dressings) and (iv) dipping seedlings in Zn solutions or suspensions at transplanting (e.g. rice; Slaton et al. 2001). Applications of manures to soils can also alleviate Zn deficiency of plants (Srivastava and Sethi 1981). Several other fertiliser practices also influence plant Zn status. For example, in Australia, the level of Zn impurity in different macronutrient fertilisers varies widely from about 400-600 mg/kg in single superphosphate to about 70 mg/kg in diammonium phosphate (Tiller 1983; Brennan and Gartrell 1981; Brennan 1986). Depending on the rate and frequency of fertiliser use, the impurity of Zn in fertilisers can supply significant amounts of Zn for plants grown on soils of low Zn status (Ozanne et al. 1965; Riley et al. 1992). Indeed, the incidence and severity of Zn disorders (including crop failures) increased in the 1980's in many areas of southern Australia when low-Zn, high analysis NP fertilisers, replaced single superphosphate applications (Brennan and Gartrell 1981; Brennan 1986).

Agronomic effectiveness: Mortvedt and Gilkes (1993) have recently reviewed the effectiveness of various Zn sources, ranging from inorganic sources to Zn in compound granulated fertilisers. The effectiveness of the Zn source depends on the water solubility and whether the form is as a granule or a fine powder. For example, ZnO is ineffective in the granular form because the decreased specific surface of the granular fertiliser with its low solubility in water (Mortvedt 1991). Giordano and Mortvedt (1972), Mortvedt (1991) and Martens and Westermann (1991) also discuss the agronomic effectiveness of micronutrients, including Zn, in macronutrient fertilisers. Various workers have studied the movement of Zn out of fertiliser granules (Mortvedt and Giordano 1967; Hossner and Blancher 1969; Giordano and Mortvedt 1969; Mortvedt and Giordano 1969a, b;
Giordano *et al.* 1971; Gilkes *et al.* 1975; Gilkes 1977; Giordano and Mortvedt 1978; Gilkes and Sadlier 1981) and found minimal movement of Zn out of the granule with a small volume of soil adjacent to the granule enriched with Zn. As there is minimal movement of Zn out of granular fertiliser, the placement of Zn fertiliser for root contact and uptake by plants often affects the agronomic effectiveness (Giordano *et al.* 1966; Hawkins *et al.* 1973). The reaction of Zn in various carrier fertiliser compounds on the availability of Zn to plants (Jackson *et al.* 1962; Ellis *et al.* 1965; Allen and Terman 1966; Giordano and Mortvedt 1972; Lehr 1972). For example, the effectiveness of Zn in granulated NPK fertilizers does depend on the form of the carrier, with much less Zn being provided by DAP relative to MAP and superphosphate.

### 1.7.2 Edaphic constraints

Zinc uptake may be reduced and symptoms of Zn deficiency may be induced or intensified by edaphic constraints that restrict root development. For example, root disease may limit Zn uptake *per se*, but the leakage of carbohydrates and/or amino acids into the rhizosphere of Zn deficient plants (Loneragan *et al.* 1987) may encourage greater invasion by soil pathogens (Graham 1983; Graham and Webb 1991). Indeed, the exacerbating effects of herbicide applications on the severity of root diseases (Rovira and McDonald 1986) and on reducing nutrient uptake in plants, particularly Zn (Rudgers *et al.* 1970; Robson and Snowball 1989, 1990; Osborne and Robson 1992; Osborne *et al.* 1993) are concerns for conservation farming systems where greater reliance is placed on the use of herbicides for weed control. Studies have found that applications of diclofop-methyl and chlorsulfuron to a Zn deficient sandy soil intensified Zn deficiency in wheat and induced the disorder on soils of near-adequate soil Zn (Robson and Snowball 1989, 1990; McLay and Robson 1992). The major effect of these herbicides on the Zn status of plants was via effects of decreased root growth, decreased root extension, increased root thickness and as a consequence of root morphology effects there was a decreased volume
of soil explored by roots for Zn uptake (Robson and Snowball 1989, 1990; McLay and Robson 1992; Longnecker and Robson 1993; Dong et al. 1995).

1.7.3 Rotation effects

Numerous examples are available on the effect that Zn deficiency has on reducing yield of individual crops, but Zn deficiency may also limit productivity in other ways (e.g. Leggett and Westermann 1986). Firstly, where pasture legumes or pulse crops are part of rotation sequences, nodule size and their proliferation on roots of legume hosts are known to be restricted by Zn deficiency (e.g. Reuter et al. 1982a). Also, the suppressed shoot and root growth further limits the quantity of symbiotically-derived N accrued during the legume phase (determined by the density and vigor of the legume) and its availability for subsequent crops. The interaction between Zn requirement of crop species and soil N supply derived from pasture legumes requires further research in medium-term (4–6 years) field experiments.

Secondly, Zn deficiency has been implicated in the 'long fallow disorder' observed in a range of crops, especially linseed, grown on Vertisols after long periods of uncultivated fallow (> 12 months duration) in northern grain areas of Australia. The 'long fallow disorder' has been associated with declines in viable VAM propagules during fallowing and the subsequent development of P and possibly Zn deficiencies (Thompson 1987). In mycorrhizal plants, P deficiency symptoms are eliminated and plant Zn status improved (Lambert et al. 1979; Thompson 1990; Ryan et al. 2002; Ryan and Angus 2003). Similar responses have been obtained in peanuts grown on an oxisol (Bell et al. 1989).

1.8 Decline in availability of Zn with time

Fertiliser Zn applied to soil not only provides Zn for plant uptake in the year of application but in the years after application. Zinc supplied to plants in the years after
application is referred to here as the residual value of the fertiliser. Knowledge of the residual value of Zn fertiliser is important because it determines how long an application of fertiliser Zn provides adequate Zn for plant production and when a further application of fertiliser Zn is required to prevent Zn deficiency reducing plant yields and production. To estimate the residual value of Zn fertiliser it is necessary to know the soil properties that control Zn reactions and determine the availability of Zn within the soil.

Brummer et al. (1988) suggested that the adsorption of Zn (and other heavy metals) by goethite was determined by three steps: adsorption on external surfaces, solid state diffusion of metals from external to internal sites and fixation within the particles. The diffusion of Zn to internal binding sites of goethite increases with time, temperature and Zn concentration (Brummer et al. 1988). Thus, Brummer et al. (1988) postulated diffusion of Zn into goethite is one possible reason why the availability of Zn (and other heavy metals) declines with time. Other soil properties contribute to the decline in availability and have been reviewed in preceding sections (Chapter 1.3.5). The factors which lead to a decline in the residual value of Zn will be discussed in the following section.

1.8.1 Mechanisms of the decline in the availability of Zn with time

Reactions of applied Zn with soil constituents appear to be the major cause of the reduction of Zn availability with time since the capacity of soils to adsorb Zn greatly exceeds the usual amounts of Zn applied. The mobility of Zn has been reported to vary markedly for soils of different texture. For example, Jurinak and Thorne (1955) found that Zn applied, as a soluble chloride source, to the surface of a silty clay moved <3 cm under strongly leaching conditions. Zinc that was applied as either the oxide (insoluble source) or sulfate (soluble source) did not move either in columns of a sandy loam or a silt loam soil that were leached with water (Brown et al. 1962). Moreover, radioisotope
studies have shown that most of the Zn applied was retained in the upper 3 cm of loamy sand, although there was movement of Zn to a soil depth of 12 to 18 cm depending on the soil texture (Singh 1974). In contrast, Barrows et al. (1960) measured Zn movement to a maximum depth of 45 cm in a sandy soil. Radioisotope $^{65}$Zn applied to the surface of a sandy loam was leached to 30 cm by the equivalent of 114 mm of surface water; however, the highest concentration was in the upper 4 cm (Abebe 1972). Hence whilst redistribution of Zn in the soil, especially in sandy soils, has been studied, the extent of leaching losses of Zn from the root zone on the sandy soils has not been determined.

Removal of Zn may occur by physical removal of soil during erosion but losses in crop products has been shown to be small for WA. Typical removal of Zn in products is; wheat, barley about 5-25 g Zn/t of grain; lupin seed 17-30 g Zn/t and wool and animal meats <10g Zn/ha (see Chapter 7.2 & Chapter 7.3). However, losses in crop products for other farming systems can be very high. For example, in the high yielding wheat-rice rotations of Asia losses of Zn in crop removal is about 250 g Zn/ha in the grain of the wheat and rice crops each year (Bell et al. 2004).

1.8.2 Observations of decline in availability of Zn in glasshouse and field studies

In a field experiment, after Zn fertiliser application of 11.2 kg Zn/ha, DTPA and HCl extracted Zn from soil decreased rapidly in the first year, followed by a more gradual decrease in years 2 and 3, and then was virtually constant in years 4 and 5 (Boawn 1974). Using a defined critical soil extractable Zn concentration (soil test value), the lengths of time that different amounts of applied Zn were able to maintain soil test Zn values at a concentration sufficient for plant growth were defined. A Zn application of 11.2 kg/ha maintained soil test Zn above the critical concentration for 1 year in a calcareous soil and for 3 years in a silty loam. Brown et al. (1964) showed that Zn applied to a sandy loam at 2 kg Zn/ha, the Zn in the soil was converted into forms that could not be extracted by 0.1
mol/L HCl at a rate of about 0.3 mg Zn/kg/yr. Where 18 kg Zn/ha had been applied to the soil, the applied Zn was converted to non-extractable forms by 0.1 mol/L HCl at a rate of about 1 mg Zn/kg/yr (Brown et al. 1964).

In a greenhouse study with 6 soil types, the residual value of applied Zn as measured by soil extractants and yield of successive corn crops, varied widely with soil type (Brown et al. 1964). Concentrations of dithizone-extractable soil Zn decreased rapidly with time in all soils especially for the first crops and the decrease in extractable Zn for subsequent crops was slower. Tiller et al. (1972) noted that EDTA-extractable Zn decreased during a pot experiment, with the decrease in EDTA-extractable Zn most rapid in soils with a high soil pH value. The decline in EDTA-extractable Zn could not be explained by the removal of Zn from the soil by plants.

Similarly, Kuo and Mikkelson (1980) showed for 6 soil types that the concentrations of DTPA-extractable Zn decreased rapidly during the first few weeks and then declined more slowly with time. Kuo and Mikkelson (1980) suggested that diffusion was not the only mechanism regulating the rate of Zn desorption by DTPA: a result in agreement with the results of Barrow (1986b) and Brummer et al. (1988).

Zinc fertiliser applied to soils of WA for cereal and pasture production has been suggested to have a long residual value (Anon. 1961). However, no data on length of time that the application of Zn remains fully effective for yield are available and residual effectiveness of Zn fertiliser is a topic of further study in this thesis (Chapters 6 & 7).

1.9 Aims of this thesis

This review has shown that low Zn is a widespread problem for many soils in WA, and subterranean clover and wheat are the major pasture and crop species respectively in the
This thesis determined the effectiveness of freshly applied Zn for clover and wheat for divergent soils collected in WA and Australia, and the yield increases were related to soil properties to determine which properties predicted plant yield responses to freshly applied Zn.

The thesis then compared the effectiveness of Zn chelate and Zn sulfate applied in small amounts in solution to the canopy of wheat grown in field experiments. This was to determine if a Zn deficiency recognised in a growing wheat crop could be treated with a Zn foliar spray to reduce or prevent grain yield decreases at harvest. The experiments determined which source of Zn spray, the amount and timing were appropriate to reduce grain yield loss due to Zn deficiency in the soil.

Experiments reported in the thesis quantify the residual value (RV) of Zn fertiliser applied to the soil for clover and wheat. This was done in glasshouse and field studies when the RV was determined using plant yield, Zn content in plant tissue and soil test Zn by DTPA and ammonium oxalate extraction. In addition, critical soil and plant test values were determined as tools to diagnose and predict Zn deficiency. In the glasshouse study, many diverse soils from Australia were used and the RV’s were related to soil properties to assess which soil properties adequately predicted the RV. In both the field and glasshouse RV studies, critical soil test Zn was determined as a predictor of the need to reapply fertiliser Zn.

Several crop species (canola, various lupin and pulse species) are now grown in rotation with wheat in WA, and the Zn requirement of the new species is poorly understood or not known. Therefore glasshouse and field studies were completed to compare the Zn requirement of the new crop species with wheat, using plant yield and critical values in young leaves and the results are reported in this thesis.
The thesis is organised into seven chapters, appendices and a reference section. Chapters 2 to 6 describe how the experiments were done, and the results and discussion of each experiment are presented. Chapter 7 presents a general discussion of the findings.
Chapter 2

Reaction of Zinc with Soil and its Availability to Plants.

2.1 Subterranean clover

2.1.1 Abstract

The need for Zn fertiliser can be predicted by soil testing. However, the reliability of a soil test is diminished if the critical levels vary with soil properties. Five glasshouse experiments were conducted in which clover (*Trifolium subterraneum* L.) cv. Nungarin was grown in pots of a wide range of Australian soils to which Zn was added. Levels of Zn extractable in DTPA and in ammonium oxalate were determined and related to the growth of clover. DPTA extractable Zn ($Zn_{DPTA}$) was found to be a reliable predictor of the response of clover to Zn added to the soils whereas ammonium oxalate extractable Zn was not. However, the critical levels of $Zn_{DPTA}$, determined for the maximum growth of clover was found to vary markedly among the soil types. The $Zn_{DPTA}$ critical levels for the soils were related to the clay content (%) and to pH (1:5 water) of the soil. Considering all 54 Australian soils, the stepwise linear regression found to predict critical DTPA Zn was:

$$Y (DTPA \text{ critical level, mg/kg}) = -0.019 + 0.034 \, pH_{Ca} + 0.006 \, \text{clay (\%)} \quad R^2 = 0.93$$

For the non-responsive soils in the pot experiments, DTPA extractable Zn was always above the calculated critical $Zn_{DPTA}$ level. Hence when the $Zn_{DTPA}$ was adjusted for soil pH and clay content, the critical concentration reliably distinguished Zn responsive from non-responsive soils.

The critical Zn concentration for YOB at 30 days after emergence was found to be about 12 mg/kg. At flowering, there was a greater spread of data points for each Zn concentration measured in plant parts so that a wide critical concentration range (varied from 14 to 23 mg Zn/kg) was determined. Similarly, Zn concentration in whole shoots related to relative yield of shoots resulted in a wide range of values (9 to 16 mg Zn/kg) at which 90% of the maximum yield was reached.

2.1.2 Introduction

Zinc is an essential micronutrient required for the growth of pasture and cereal plants (Donald and Prescott 1975; Mengel and Kirby 1978; Brown et al. 1993). Zinc deficiency of plants is widespread. It has been estimated that about 30% of the agricultural soils of the world may be Zn deficient for the normal growth and development of a wide range of crop and pasture plants (Sillanpää 1982).

Many Australian soils are naturally low in Zn (Donald and Prescott 1975). In WA, a Zn application between 0.5 and 1.5 kg Zn/ha (Gartrell and Glencross 1968) is required for profitable agricultural production. Fertiliser practice has been concerned with maintaining soil Zn supply at levels adequate for maximum production of *Trifolium subterraneum* L. (henceforth clover) for the pasture phase of a pasture/wheat rotation. With the contamination of Zn (400-600 mg/kg) in single superphosphate (9.1 % P, 10 % S, 20 % Ca; henceforth superphosphate) made from Nauru and Christmas Island rock phosphates (Walkley 1940; Bingham 1959; Williams 1974), the initial application of Zn remained sufficient for many years (Anon. 1961) providing at least 150 kg/ha of superphosphate is applied annually (Brennan and Gartrell 1981). However, in recent years the application of superphosphate has declined below 150 kg/ha annually on many WA soils. Consequently, it has become necessary to characterise the Zn status of soils used for cereal crop and pasture production. The increased usage of imported
diammonium phosphate (DAP) fertiliser for cropping, which supplies about one twelfth of the Zn in superphosphate, has often resulted in the immediate occurrence of Zn deficiency in the crop and subsequent pastures (Brennan and Gartrell 1981). This change in fertiliser management created further need to characterise the Zn status of WA soils.

In this study, the glasshouse work investigated the effectiveness of two soil extractants for Zn in predicting the response of clover yields on a range of Australian soils when Zn fertiliser was added either before or after an incubation treatment. A range of Australian soils were selected to calibrate the soil test for Zn across a range of soil properties (e.g. higher clay% soils than found in WA soils).

2.1.3 Materials and Methods

**Experimental Design:** Five experiments were conducted using a range of Australian soil types. All experiments included the same two responsive soils from WA. A total of 54 different soils from Australia were used (Appendix 1).

In all experiments, there were three replicates of each treatment and the design was a completely randomised factorial combination of: (a) soils; (b) Zn application (0, 400, 800 µg Zn/3 kg soil); (c) time of Zn application (either before or after incubation).

**General Procedures:** Bulk samples from the surface (0-10 cm) of all soils were used for the experiments. There were 43 soils selected within WA, three soils from South Australia (Nos. 8, 41, 53-Table 2.1), five soils from Queensland (Nos. 28, 48, 50, 52, 54 - Table 2.1) and three soils from Victoria (Nos. 19, 38, 51-Table 2.1). Soil collection sites and names are listed in Appendix 1.

Soil pH (pH_{Ca}) was determined on a 1/5 w/v soil:0.01 M CaCl₂ suspension (Rayment and Higginson 1992). Total Zn concentration was determined by atomic absorption spectrophotometry after digestion by perchloric and hydrofluoric acids. Clay, sand and
silt percentages were determined by mechanical analysis (Day 1965). Sesquioxides were
determined using a modified Coffin procedure (Hesse 1971). Organic carbon (Walkley
and Black 1934), ammonium oxalate Zn (Gupta and Mackay 1966), DTPA extractable Zn
(Lindsay and Norvell 1978) and bicarbonate extractable soil phosphorus (Colwell 1963)
were also determined for each soil. The percentage free calcium carbonate (Hesse 1971)
of soils No. 38, 40, 48, 50, 51, 52, 53 and 54 of Table 2.1 was 0.7, 5.8, 3.0, 4.0, 5.0, 3.0,
10.0 and 4.0 % respectively: other soils contained no detectable CaCO₃.

**Glasshouse Techniques:** All soil types were air dried and sieved through a 3.86 mm
stainless steel sieve, and 3 kg aliquots of soil weighed into 16.5 cm (surface diameter)
undrained plastic pots lined with polyethylene bags.

A basal dressing of nutrients in mg/pot (K₂SO₄, 328; MnSO₄.4H₂O, 53; Na₂MoO₄.2H₂O,
0.8; CuSO₄.5H₂O, 14; CoSO₄.7H₂O, 0.86; H₃BO₃. 1.0; MgSO₄.7H₂O, 64; CaCl₂.2H₂O,
300) was applied in solution to the soil surface. On a surface area basis 214 mg/pot is
equivalent to 100 kg/ha. Macronutrient salts were purified using dithizone-carbon
tetrachloride (Hewitt 1966). Basal phosphorus (P) solution as KH₂PO₄ was applied at
different levels to each soil. The amounts of P were determined for each soil in previous
experiments that involved incubation when all other nutrients, including Zn, were non-
limiting to achieve maximum dry matter production of clover. Zinc was applied in
solution (5 ml/pot) as Zn sulfate where appropriate. After all applied solutions had dried
the contents of each pot were thoroughly mixed by shaking in a plastic bag. To minimise
contamination, the mixing was carried out by progressively working from the nil Zn
treatment to the highest level of applied Zn and changing plastic bags between soils.

Zinc was applied before or after incubation. For incubation, all pots were watered to field
capacity using de-ionized water and incubated in controlled temperature water baths at
30°C for 30 days. Sealing each pot of soil in polyethylene bags prevented water loss from
the pots. After incubation, all soils were air dried before Zn solutions were added to those pots receiving Zn after the incubation. The soils in each pot were again thoroughly mixed as outlined above.

Soil samples (100 g/pot) were taken from each pot in treatment order using a stainless steel tube. Ammonium oxalate extractable Zn, Zn$_{\text{DTPA}}$ and total Zn were determined in these samples.

Subterranean clover (cv. Nungarin) seed was inoculated with *Rhizobium trifolii* strain Wu95, lime pelleted and sown at 25 seeds per pot before thinning to five seedlings about 30 days after seedling emergence. The thinned seedlings were separated into youngest open blades (YOB) and remainder of the shoots, oven dried at 70° C for 48 hr and analysed for Zn (as outlined below). For the first 14 days, the soils were maintained near 75 % of field capacity by frequent weighing and watering with deionised water. All experiments were conducted between April and November. During the growth of plants, regular watering with deionised H$_2$O kept the soil moisture near field capacity. At mid to late flowering all the plants were harvested and washed, and the YOB were separated from the remainder of the shoots. The plant shoots and YOB were then oven-dried at 70°C for 48h and weighed. Replicate samples were individually digested in a nitric-perchloric acid mixture (Johnson and Ulrich 1959) and analysed for Zn by atomic absorption spectrophotometry (Allan 1961). Standard samples were included in each batch of samples that were analysed for quality control of the Zn analysis.

**Analysis of data:** All data were analysed by standard analysis of variance. Ammonium oxalate extractable Zn often gave a different relationship between the soil extractable Zn concentration and the yield of clover shoots, one for freshly applied and another for the incubated Zn. That is, a single soil value of ammonium oxalate extractable Zn was associated with differing relative yields (see Figure 2.5). On other soils there was a single
soil test calibration for ammonium oxalate was determined. These variations in the calibration relationships between soil test value and yield frequently encountered for ammonium oxalate render it of little use for a soil test (see discussion in literature review; Chapter 1.5.6). Hence, ammonium oxalate extractable Zn was not pursued further in defining critical soil test concentration for clover yield. Hand-fitted curves were used to derive critical DTPA Zn concentrations in soils, and the critical values were confirmed using the Cate and Nelson procedure (Cate and Nelson 1965, 1971). In addition, simple linear regression and stepwise multiple regression were performed for relationships between measured soil test Zn\textsubscript{DTPA} and soil properties using standard statistical techniques. The independent variables were selected for the stepwise regression analysis based on the correlation matrix (Appendix 2) (r values) for each independent variable. Independent variables were deleted from the multiple linear regression analysis (subtractive stepwise) by reference to the "t" statistic (Zar 1984). Confidence limits (95 \% confidence) for the Zn\textsubscript{DTPA} values in the regression analysis were determined for all points on the regression line by reference to the “t” statistic, resulting in a confidence band about the regression line (see Zar (1984) Chapter 17). To ensure that Zn fertiliser is recommended where needed and to ensure no crop losses in yield from Zn deficiency are incurred, soil Zn\textsubscript{DTPA} recommendations are for the upper confidence limit called the prediction limit (Zar 1984). That is, if a critical Zn\textsubscript{DTPA} level is calculated from the selected multiple linear regression equation the confidence limit is added to that value to determine if Zn fertiliser needs to be applied. If the measured Zn\textsubscript{DTPA} value is less than the model calculated values, Zn fertiliser is required.

The relationship between dry matter production (dependent variable, y-axis) and the concentration of Zn in the YOB and or rest of shoots (independent variable, X-axis) was used to define critical Zn concentrations in plant parts (Ohki 1977; Ware \textit{et al.} 1982; Reuter and Robinson 1997). The Mitscherlich equation is often used to define the
relationship between Zn concentration in plant parts and dry matter yield of shoots (Ware et al. 1982). The critical value is the Zn concentration in the plant part that was related to 90% of the maximum yield (Ulrich and Hills 1967; Reuter et al. 1997a, b). For plant testing, to reduce variations in plant yields due to different soil types, yield was expressed as a percentage of the maximum (relative) Zn non-limiting yield (Smith and Loneragan 1997). Maximum yield plateaus were clearly defined for the relationship between plant yield and the amount of freshly or incubated Zn applied. Consequently, the relationship between absolute yield for highest level of Zn freshly applied was used to calculate the relative yield for use in the relationship between plant yield and Zn concentration in tissue. Hand-fitted curves to the relationship between yield and the concentration of elements in tissue often give satisfactory descriptions of the data and have been recommended for deriving critical concentrations values for elements in plant tissue (Reuter 1980; Reuter et al. 1983; Wilhelm et al. 1993; Khan et al. 1998). Therefore, hand-fitted curves to derive critical Zn concentrations in remainder of shoots and YOB were used, and the results were confirmed using the Cate and Nelson procedure (Cate and Nelson 1965, 1971).

2.1.4 Results

Symptoms of Zn deficiency in clover plants (Reuter 1980; Brennan and Gartrell 1981, Reuter et al. 1982a, b) were readily recognised on many soils about 17 to 21 days after emergence of the seedlings. The severity of the Zn deficiency symptoms and the magnitude of the growth response of clover to added Zn varied among soils. The symptoms of Zn deficiency were as described previously for other cultivars of clover (Rossiter 1951; Millikan 1953; Carroll and Loneragan 1968; Reuter et al. 1982a, b). Zinc deficiency first appeared on the youngest leaves. Zinc deficiency decreased leaf blade expansion and delayed the development of trifoliate blades and branches. Zinc deficient clover plants were stunted and rosetted.
Table 2.1. Properties of soils used in glasshouse experiments. Also listed are the confidence interval (CI)
about the predicted DTPA Zn value (Ŷ).
Total Extractable Zn
DTPA Critical Level
No pHCa Sand Clay OC Al2O3 Fe2O3 Zn DTPAa NH4OXb Observedc Predictedd CI [Ŷ]e
1:5
%
%
%
%
% mg/kg mg/kg mg/kg
mg/kg
mg/kg mg/kg
5.2 97.0 1.5 2.58 0.04 0.02
4.2
0.06
0.12
0.14
0.17
0.012
1
5.1 98.0 2.0 0.62 0.02 0.02
1.0
0.06
0.14
0.19
0.17
0.012
2
5.1 96.0 2.0 0.42 0.02 0.06
1.6
0.05
0.12
0.14
0.17
0 012
3
4.5 94.0 2.5 0.39 0.02 0.08
1.4
0.06
0.09
0.18
0.15
0.012
4
4.4 93.0 2.5 0.40 0.02 0.08
1.8
0.05
0.13
0.14
0.15
0.016
5
5.2 95.0 3.0 0.87 0.18 0.27
1.6
0.02
0.13
0.18
0.17
0.015
6
5.1 97.0 3.0 0.64 0.03 0.07
1.1
0.10
0.21
0.20
0.17
0.012
7
5.1 96.0 3.0 0.66 0.01 0.06
1.4
0.10
0.14
0.15
0.17
0.012
8
5.3 96.0 3.5 0.63 0.07 0.25
1.1
0.05
0.07
0.20
0.18
0.011
9
1.4
0.09
0.14
0.17
0.18
NR
10 5.2 95.0 3.5 0.62 0.02 0.03
2.1
0.27
0.48
0.00
0.16
0.011
11 4.6 94.0 3.5 1.47 0.06 0.02
3.1
0.07
0.13
0.16
0.18
0.011
12 5.1 96.0 4.0 0.43 0.17 0.62
2.3
0.06
0.23
0.15
0.19
0.012
13 5.5 94.0 4.0 0.59 0.08 0.43
9.4
0.05
0.11
0.18
0.21
0.011
14 6.2 88.0 4.0 4.70 0.90 1.50
5.7
1.33
3.03
0.00
0.20
NRf
15 5.8 95.5 4.0 0.41 0.10 0.30
2.3
0.07
0.50
0.15
0.15
0.019
16 4.1 94.0 4.5 4.04 0.22 1.70
4.2
0.23
0.60
0.00
0.17
NR
17 4.8 84.5 4.5 2.72 0.15 0.41
1.6
0.07
0.14
0.21
0.18
0.012
18 5.1 94.0 5.0 0.41 0.07 0.34
6.2
0.05
0.19
0.15
0.19
0.011
19 5.2 90.0 5.0 1.26 0.16 0.47
6.2
0.05
0.19
0.15
0.19
0.011
20 5.2 90.0 5.0 1.26 1.60 0.47
2.3
0.23
0.00
0.28
0.25
0.010
21 7.0 91.5 5.5 2.77 0.05 0.23
0.09
0.93
0.21
0.19
0.015
22 5.2 88.0 5.5 3.08 0.60 0.80 11.0
7.5
0.11
0.47
0.20
0.23
0.010
23 6.3 93.5 5.5 0.39 0.06 0.23
5.2
0.10
0.60
0.22
0.25
0.010
24 6.8 92.0 5.5 3.67 0.22 0.50
9.2
0.03
0.06
0.19
0.23
0.010
25 6.3 89.0 5.5 1.99 0.80 1.80
3.8
0.13
0.43
0.22
0.25
0.011
26 7.0 93.0 6.0 0.74 0.04 0.09
2.0
0.07
0.13
0.19
0.20
0.017
27 5.3 94.0 6.0 0.40 0.08 0.42
2.0
0.05
0.17
0.21
0.20
0.010
28 5.4 93.0 6.5 0.52 0.37 1.40
3.5
0.06
0.12
0.20
0.21
0.011
29 5.6 91.0 6.5 0.66 0.05 0.18
1.7
0.03
0.12
0.14
0.18
0.009
30 4.6 93.0 6.5 0.45 0.09 0.33
0.18
0.87
0.20
0.22
0.024
31 5.2 82.0 9.5 1.68 0.45 0.22 39.0
4.6
0.04
0.18
0.16
0.23
NR
32 5.4 82.0 10.0 0.72 0.25 1.20
0.23
1.75
0.23
0.23
NR
33 5.5 77.5 11.0 0.88 0.08 0.40 23.0
3.9
0.09
0.26
0.25
0.26
0.011
34 6.3 91.0 11.0 0.78 0.08 0.26
6.7
0.15
0.58
0.21
0.20
0.015
35 4.6 85.0 11.0 1.02 0.30 1.80
3.7
0.12
0.52
0.21
0.23
NR
36 5.3 85.0 12.0 0.83 0.48 2.30
0.40
3.30
0.00
0.26
0.014
37 6.2 77.0 12.0 1.98 0.16 1.80 28.0
0.15
1.53
0.24
0.25
0.020
38 5.8 80.5 12.5 0.40 0.10 2.30 51.0
0.17
1.00
0.25
0.28
0.028
39 6.6 50.0 13.0 1.44 0.16 1.20 28.0
4.5
0.05
0.14
0.15
0.20
0.021
40 4.1 84.0 14.0 0.61 0.16 0.71
8.5
0.26
0.71
0.00
0.26
0.012
41 5.5 80.0 16.0 0.63 0.08 0.56
0.09
0.30
0.40
0.35
0.012
42 7.4 64.0 20.0 1.28 0.16 0.40 10.0
0.27
1.13
0.00
0.29
0.012
43 5.7 74.0 20.0 1.62 0.12 0.86 16.0
8.2
0.19
0.50
0.00
0.29
NR
44 5.7 70.0 20.0 0.75 0.06 0.47
0.17
0.69
0.28
0.32
0.014
45 6.0 70.0 22.0 3.22 0.15 0.66 14.0
0.35
1.24
0.00
0.31
0.019
46 5.3 66.0 25.0 0.44 0.20 4.00 26.0
6.6
0.09
0.60
0.42
0.40
0.020
47 7.5 66.0 27.0 0.84 0.10 0.22
0.23
2.43
0.45
0.46
0.020
48 7.2 47.0 39.0 1.50 0.55 2.20 47.0
0.23
1.23
0.46
0.48
0.024
49 6.5 37.5 45.5 0.26 0.20 1.60 26.0
0.14
1.75
0.51
0.51
0.023
50 7.4 37.0 46.0 0.62 0.32 0.57 32.0
0.20
4.80
0.50
0.51
0.025
51 7.4 26.0 46.0 1.37 0.46 3.40 98.0
0.22
2.46
0.54
0.52
0.026
52 6.9 30.0 50.0 1.14 0.21 0.48 58.0
1.47
0.00
NR
0.53
NR
53 7.2 32.0 51.0 1.34 0.28 0.62 42.0
0.21
0.45
0.52
0.58
0.032
54 7.1 23.0 59.0 1.09 0.37 1.00 64.0
a
DTPA soil extractable Zn from the nil Zn treatment.
b
The ammonium oxalate soil extractable Zn from the nil Zn treatment.
c
d
Predicted DTPA Zn concentration from the fitted stepwise multiple linear regression.
e
Confidence interval about the predicted critical DTPA values (Y) using methods outlined in Zar (1984).
f
NR is no response; added Zn did not increase the dried weight of shoots.

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**Dry matter production:** Zinc application did not increase dry weight of shoots of clover on eight of the soils (Soil No. 10, 15, 17, 32, 33, 36, 44, 53 - Table 2.1). Dry matter production increases of 5 to 25 % to applied Zn were measured for 20 soils on which clover grew without any visual symptoms of Zn deficiency. On all soils where there was an increase in dry matter production of clover shoots to applied Zn, the incubation treatment decreased the dry weight of shoots on the intermediate Zn level (400 μg Zn/pot). For the highest Zn level (800 μg/pot), the incubation treatment decreased the dry matter production on several soil types (6, 9, 13, 14, 19, 21, 22, 24, 28, 31, 32, 39, 42, 47, 48, 50, 51, 52, 53, 54 - of Table 2.1). That is, the incubation decreased the availability of soil applied Zn for growth of clover. Examples of the effects of incubation and Zn on clover growth are shown for selected soils (Table 2.2).

**Table 2.2.** The effect of incubation and Zn application on the dry weight (g/plant) of clover shoots on selected soils reported in Appendix 1.

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Zn application (µg/pot)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>F</td>
</tr>
<tr>
<td>2</td>
<td>0.23</td>
</tr>
<tr>
<td>12</td>
<td>0.12</td>
</tr>
<tr>
<td>19</td>
<td>0.25</td>
</tr>
<tr>
<td>24</td>
<td>0.35</td>
</tr>
<tr>
<td>57</td>
<td>0.50</td>
</tr>
</tbody>
</table>

*F is the freshly applied Zn: applied after the incubation treatment.*

*I is the incubated Zn applied immediately before the incubation treatment.*

**Zinc concentration in plant parts related to dry matter production:** There was a good relationship between Zn concentration in the YOB and the relative yield of clover shoots at 30 days. The critical Zn concentration for YOB at 30 days after emergence was found to be about 12 mg/kg (Figure 2.1). At flowering, there was a greater spread of data points for each Zn concentration measured in plant parts so that a wide critical concentration range (varied from 14 to 23 mg Zn/kg) could be determined (Figure 2.2).
**Figure 2.1** The relationship between the relative yield of clover shoots (% of maximum for each soil) grown on the responsive soils and Zn concentration in youngest opened blades (YOB) (mg/kg) sampled at 30 days after seedling emergence.

**Figure 2.2** The relationship between the relative yield of clover shoots (% of maximum for each soil) grown on the responsive soils and Zn concentration in youngest open blades (YOB) (mg/kg) sampled at mid-flowering.
The relationship between the relative yield of clover shoots grown on the responsive soils and Zn concentration in whole shoots (mg/kg) sampled at 30 days after seedling emergence. Similarly, Zn concentration in whole shoots related to relative yield of shoots resulted in a wide range of values (9 to 16 mg Zn/kg) at which 90 % of the maximum yield was reached (Figure 2.3).

Soil Extractable Zn: The extraction of soil Zn by ammonium oxalate reflected Zn additions (Figure 2.4). The incubation treatment did not always lower the extractable Zn (Figure 2.4). For example in soil 21 (Figure 2.4), the Zn extracted by ammonium oxalate was similar (about 0.22 mg Zn/kg) for both the freshly added Zn and incubated Zn in the moist soil, resulting in a dual relationship between extractable Zn and dry matter production of clover shoots (Figure 2.4). The extraction of soil Zn by DTPA always reflected the addition of applied Zn and consistently showed the effect of the incubation treatment in lowering the availability of applied Zn (Figure 2.5).
Figure 2.4 The relationship between the relative yield for dried shoots and ammonium oxalate soil extractable Zn (mg/kg) for both the freshly applied and incubated Zn for a sand (soil 21; ◇◆) and a sandy clay loam (soil 35; △▲). Dashed lines represent 90% of the maximum yield from which critical levels (indicated by vertical arrows) of ammonium oxalate extractable Zn were determined. Freshly applied Zn open symbols; closed symbols incubated Zn.

Figure 2.5 The relationship between DTPA soil extractable Zn (mg/kg) for both the freshly applied and incubated Zn and the relative yield for dried of shoots (%) for a sand (soil 21; ◇◆) and a sandy clay loam (soil 35; △▲). Dashed line represents 90% of the maximum yield from which critical levels (indicated by vertical arrows) of DTPA Zn were determined. Freshly applied Zn open symbols; closed symbols incubated Zn.
The relationship between dry matter production and $Zn_{DTPA}$ for responsive soil types gave a range of critical $Zn_{DTPA}$ values that varied greatly among the soils (Table 2.1).

The critical value of $Zn_{DTPA}$ required for 90% of maximum dry matter production of clover shoots was not closely related by simple correlation to soil properties apart from clay content (Figure 2.6) and to a lesser extent soil $pH_{Ca}$ values (Figure 2.7).

![Figure 2.6](image)  
**Figure 2.6** The simple linear relationship between DTPA soil critical level (mg/kg) and clay content of the 46 Australian soils which were Zn responsive for clover.

The two important soil properties, $pH_{Ca}$ and clay (%) were selected from a stepwise regression analysis based on the critical 't' value for each independent variable. The deletion of the soil property with the lowest 't' value in the selected multiple regression equation (Table 2.3) had a marked effect on the multiple regression equation by decreasing the coefficient of determination ($R^2$) (Table 2.3).

The selected model was:

$$
\hat{Y} (DTPA \, critical \, level) = -0.019 + 0.034 \, pH_{Ca} + 0.006 \, clay \, (\%) . \quad (1)
$$
where $\hat{Y}$ is the critical DTPA soil extractable Zn level (mg/kg), and has a coefficient of determination ($R^2$) of 0.94. Appropriate statistics for this model are presented in Table 2.3.

![Graph](image.png)

**Figure 2.7** The simple linear relationship between DTPA soil critical level (µg/kg) and the soil pH value measured in calcium chloride for 46 Australian soils which were Zn responsive for clover.

The inclusion of free calcium carbonate (%) improved the model ($R^2 = 0.95$), but the extra soil property is required for only eight responsive soil types containing CaCO₃ (Table 2.1).

The critical Zn$_{\text{DTPA}}$ model is then:

$$\hat{Y} \ (\text{critical Zn}_{\text{DTPA}}) = 0.051 + 0.02 \ pH_{Ca} + 0.005 \ \text{clay} \ (%) + 0.018 \ \text{CaCO}_3 \ (%) \quad (2)$$
Table 2.3. Statistical values of the coefficient of determination for the relationship between critical concentration of DTPA Zn and several soil properties of soils deficient in Zn for maximal growth of clover.

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Independent Variables</th>
<th>Simple Regression</th>
<th>Multiple Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>Critical level of DTPA Extractable zinc</td>
<td>Iron oxide Organic carbon Alum. oxide pHc Ca clay</td>
<td>0.12 0.0008 0.007 0.64 0.89</td>
<td>0.95b 0.94 0.94 0.89</td>
</tr>
</tbody>
</table>

a The R² value for the relationship between the critical DTPA extractable Zn and the respective soil property.
b The R² value is for the subtractive stepwise regression including the variable in that row plus those variables in the following rows.
c Alum. oxide is aluminium oxide.

For each soil type, a confidence limit was determined for each predicted critical ZnDTPA level (Ŷ) (Table 2.1). The critical ZnDTPA levels (Ŷ) and the confidence limits (Table 2.1) for each interval of the critical Zn values (Table 2.4) were used to determine the predicted DTPA level at which Zn fertiliser would be recommended for a particular soil.

Table 2.4. Multiple linear regression model for critical Zn levels and properties of soils. Only those deficient in Zn for maximal clover growth (Equation (1)) were included in the model.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficients</th>
<th>s.e.</th>
<th>t-statistic</th>
<th>Probability</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>pHca</td>
<td>0.034</td>
<td>0.005</td>
<td>6.84</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Clay</td>
<td>0.006</td>
<td>0.003</td>
<td>13.83</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-0.019</td>
<td>0.108</td>
<td></td>
<td></td>
<td>0.94</td>
</tr>
</tbody>
</table>

The calculated confidence limit was 0.008 mg/kg for the lowest range of predicted critical ZnDTPA values (0.12 to 0.15 mg Zn/kg) and increased to 0.028 mg/kg for the highest critical ZnDTPA values (0.55 to 0.6 mg Zn/kg) determined in this study (Table 2.5). For example, if the critical ZnDTPA for a soil was 0.25 mg Zn/kg as calculated from eqn 1, the confidence limit of 0.013 (from Table 2.5) would be added to this value (about 0.26 mg Zn/kg). Hence, if the measured DTPA extractable Zn of the same soil was 0.20 mg/kg, Zn fertiliser would be recommended. Similarly, if the measured ZnDTPA was a higher value than the calculated critical ZnDTPA plus the respective confidence limit for the calculated value, no Zn fertiliser would be recommended.
Table 2.5. The confidence limits about each calculated DTPA Zn level for recommending Zn fertiliser.

<table>
<thead>
<tr>
<th>Predicted critical DTPA Zn ((\bar{Y}))</th>
<th>Confidence limit(^a) (mg/kg)</th>
<th>Predicted critical DTPA Zn ((\bar{Y}))</th>
<th>Confidence limit(^b) (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;0.12 &lt; 0.15</td>
<td>0.008(^b)</td>
<td>&gt;0.39 &lt; 0.45</td>
<td>0.021</td>
</tr>
<tr>
<td>&gt;0.15 &lt; 0.21</td>
<td>0.011</td>
<td>&gt;0.45 &lt; 0.51</td>
<td>0.023</td>
</tr>
<tr>
<td>&gt;0.21 &lt; 0.27</td>
<td>0.013</td>
<td>&gt;0.51 &lt; 0.57</td>
<td>0.026</td>
</tr>
<tr>
<td>&gt;0.27 &lt; 0.33</td>
<td>0.016</td>
<td>&gt;0.57 &lt; 0.63</td>
<td>0.028</td>
</tr>
<tr>
<td>&gt;0.33 &lt; 0.39</td>
<td>0.018</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Confidence limits for each calculated critical Zn soil test value are determined from Table 2.1.

\(^b\) The confidence limit is calculated by reference to the \(t\) distribution (\(t_{0.05} \times s.e. (\bar{Y})\)) where (\(\bar{Y}\)) is the critical DTPA-Zn for the mid-point of the each respective range of the table.

The critical levels of soil test Zn that were predicted for the eight non-responsive soils based on the multiple regression (Equation 1) were found to be less than the measured levels of Zn for each of these soils with no additional Zn (Table 2.1). That is, the multiple regression correctly identified the non-responsive soils.

2.1.5 Discussion

The relationships between soil extractable Zn, the dry matter production of clover and soil properties were investigated under glasshouse conditions. DTPA soil extractable Zn (\(Zn_{DTPA}\)) reflected Zn addition and incubation treatment, as did dry matter production of clover shoots. However, for several soils, ammonium oxalate failed to reflect the change in plant availability of Zn caused by the incubation treatment. Therefore, DTPA soil extractable Zn was a better extractant to use for predicting soils deficient in Zn for clover grown on soils from Queensland, Victoria, South Australia, the Ord region of WA and south west agricultural regions of WA. Although the DTPA soil test for Zn was developed for alkaline calcareous soils (Lindsay and Norvell 1969, 1978), it is a reliable and accurate soil test for the acid soils of WA. Similarly, Haynes and Swift (1983) concluded that the DTPA extractant was a reliable test for Zn over a wide range of soil pH values. Other studies have shown that DTPA is a reliable soil test to measure plant available Zn (Haq and Miller 1972; Lindsay and Norvell 1978; Soltanpour and Workman 1979; Brennan et al. 1993).
The major findings from this study suggest that the critical level of Zn\textsubscript{DTPA} for clover growth varied markedly with soil type from 0.13 mg/kg to 0.55 mg/kg. Such a range in critical Zn\textsubscript{DTPA} values suggests that using a single critical level of 0.5 mg/kg as suggested by Lindsay and Cox (1985) and Cox (1987) would result in unnecessary usage of Zn fertiliser on many soils of WA where the critical level is considerably lower. Numerous workers (Martens 1968; Bansal \textit{et al.} 1980; Joshi \textit{et al.} 1983; Havlin and Soltanpour 1984; Junus and Cox 1987) have correlated extractable soil Zn determined by different extractants with soil properties but few have attempted using either simple or multiple linear regressions to relate the critical levels of Zn\textsubscript{DTPA} for individual soils with measured soil properties. The critical level of Zn\textsubscript{DTPA} that was required for maximum clover dry matter production was found to vary markedly with several soil properties and was not be adequately explained by simple linear regression with any one property. However, a multiple linear regression relating the soil pH\textsubscript{Ca} and clay (\%) to the critical Zn\textsubscript{DTPA} level was successful in explaining more than 90 \% of the variation in the measured soil test value. By contrast previous researchers have attempted a separation of soils into Zn responsive and non-responsive classes using the DTPA extractable Zn (Brown \textit{et al.} 1971; Lindsay and Norvell 1978; Bansal \textit{et al.} 1980). This approach is likely to be unsatisfactory since it fails to recognise that critical levels can vary for each responsive soil. The use of a single critical level for DTPA Zn is likely to result in both false positive and false negative predictions of Zn deficiency when applied across a wide range of soil types.

Critical Zn\textsubscript{DTPA} levels determined in this study are a result of calibrating the DTPA soil test with clover dry matter production. Such a procedure has been suggested as being essential for the establishment of a reliable soil test, though often it is not performed (Cope and Evans 1985). A soil test, which reliably identifies a soil that is responsive to Zn fertiliser for clover growth would be of great benefit to farmers deciding whether Zn fertiliser should be applied. The present study further suggests that DTPA meets this criterion for prediction of
responsiveness for growth of clover. This study suggests that the critical Zn$_{\text{DTPA}}$, which was required for maximum dry weight of clover, could be determined for each soil as long as the soil properties of pH$_{\text{Ca}}$ and clay (%) are known. For soil with measured pH$_{\text{Ca}}$ values greater than about 7.5, including free CaCO$_3$ (%) improved the selected regression equation marginally (Equation 2).

The reporting of a critical concentration as a single value infers a level of precision in soil sampling and plant growth responses that is unwarranted. Hence, critical ranges are now advocated as more realistic. The critical Zn$_{\text{DTPA}}$ range can be estimated by the use of the confidence limit (see Table 2.4). Critical ranges are particularly relevant where the critical Zn$_{\text{DTPA}}$ values are near to the measured soil DTPA Zn levels (see soil Nos. 28, 32, 33 of Table 2.1). For example, soil 32 that had a critical Zn$_{\text{DTPA}}$ of 0.24 mg/kg (Table 2.1), a confidence limit of 0.013 mg/kg (Table 2.4), and therefore an upper 95% confidence level for the critical value of 0.25 mg/kg, which was below the measured Zn. It is therefore suggested that for determining when to recommend Zn fertiliser in WA the upper 95% confidence limit be added (Table 2.4) to the calculated critical Zn$_{\text{DTPA}}$ from the regression equation. The recommendation to apply Zn fertiliser would be made when the Zn$_{\text{DTPA}}$ is less than this value. This finding will allow for the separation of responsive and non-responsive soils of WA for clover growth using DTPA extractable soil Zn on a case by case basis depending on the soil properties of pH$_{\text{Ca}}$ and clay.

A close relationship was established between the concentration of Zn in the YOB and early vegetative growth of clover suggesting that the YOB was a suitable plant part test for diagnosing Zn deficiency in clover. This finding agrees with Reuter $et$ $al.$ (1982 a, b) where it was found that the critical concentration of Zn in YOB remained stable throughout the vegetative growth of clover. Both studies concluded that the critical level at 30 days was about 12 mg Zn/kg (Reuter $et$ $al.$ 1982 a, b). However, the present study did show that
sampling YOB at mid to flowering of clover resulted in an unreliable diagnosis of Zn deficiency. It is suggested that all sampling of plant parts for diagnosis of Zn deficiency in clover be done before plants start to flower. The rest of shoots were also found to be more variable relationship which led to a less reliable diagnosis of plant Zn tissue test at flowering. The commencement of flowering possibly affects the supply and translocation of Zn from older plant parts to the reproductive organs (Longneckner and Robson 1993). The movement of Zn at flowering possibly requires further research.
2.2 Wheat

2.2.1 Abstract

In a glasshouse experiment, wheat (*Triticum aestivum* cv. Gamenya) was grown in pots using a range of WA soils to which Zn fertiliser was applied. Before wheat was planted, soil samples were collected to measure DTPA soil extractable Zn (*Zn*$_{DTPA}$), which was related to the total dry matter production (straw and grain) of wheat plants.

A total of 42 soils were used for this study; 36 soils were Zn deficient and 6 soils had adequate soil Zn for wheat production. The critical *Zn*$_{DTPA}$, determined for the maximum dry matter production of wheat were found to vary among the 36 responsive soil types. For the 6 soils with adequate soil Zn, no grain yield increases to applied Zn fertiliser were obtained. For these 6 soils the calculated critical *Zn*$_{DTPA}$ values were found to be the same or below the measured soil extractable *Zn*$_{DTPA}$ where no Zn fertiliser had been applied.

For the 36 Zn deficient soils, simple linear regression indicated that critical *Zn*$_{DTPA}$ values were related to (i) soil pH (1:5 w/v 0.01 mol/L CaCl$_2$) (*pH*$_{Ca}$) ($R^2$=0.64); (ii) clay content of the soils ($R^2$=0.54), and (iii) organic carbon (OC) content of the soil ($R^2$=0.05). The critical *Zn*$_{DTPA}$ for individual soils could be predicted from the stepwise multiple regression model:

$$Y(DTPA \text{ critical Zn level}) = 0.04 + 0.019 \text{ pH}_{Ca} + 0.003 \text{ clay(\%)} + 0.004 \text{ OC (\%)} \quad (n = 36, R^2 = 0.878).$$

2.2.2 Introduction

The DTPA extractable Zn (Zn\textsubscript{DTPA}) test has been shown to be a reliable soil test for predicting Zn response of wheat crops (Bansal et al. 1980; Linday and Norvell 1978; Singh and Shukla 1985), but the critical Zn\textsubscript{DTPA} levels vary with soil type. In WA, fertiliser practice has been concerned with maintaining soil Zn at adequate levels for maximum growth and grain yield of wheat. Therefore, there was a need to relate the critical Zn\textsubscript{DTPA} levels of WA soils to soil properties so as to provide reliable fertiliser Zn advice. In this study, the glasshouse work examined the critical values of DTPA soil extractable Zn and properties of the soils that apparently influence the critical value determined for each soil. The approach was similar to that summarized in the previous section (2.1) for clover.

2.2.3 Materials and Methods

Samples from the top 10 cm of soils were collected from 42 sites in WA and used for the experiment. The soils were selected from the range of WA soils used in section 2.1. In addition to the soils of section 2.1, three soils were selected from areas were Zn deficiency has been observed and measured in wheat in the previous growing season. Two sandy soils, one from Eneabba (Soil 56- Table 2.6) and the other from Gardner River (Soil 56), and a gravelly sandy loam (soil 57) from York were collected, sieved and analysed by methods outlined in section 2.1. Selected properties of soils are listed in Table 2.6. The experimental design was a completely randomised factorial combination of 42 soils and 3 levels of Zn (0, 400, 800 µg Zn/3 kg soil). There were three replications.

General Procedures: Soil pH (pH\textsubscript{Ca}), total soil Zn concentration, clay, sand and silt percentages, sesquioxides, organic carbon and Zn\textsubscript{DTPA} were also determined for each soil by methods outlined in glasshouse techniques of Chapter 2.1.3.
**Glasshouse techniques:** Procedures of soil preparation, purification of macronutrient salts and addition of basal nutrients, minimisation of contamination of Zn, and soil sampling procedures are outlined in Chapter 2.1.3.

Pregerminated seeds of wheat (*Triticum aestivum* cv. Gamenya), with a concentration of 12 mg Zn/kg, were sown at a rate of 20 seeds per pot in mid-June. The number of plants in each pot was thinned to 10 seedlings, 14 days after emergence. At the sixth leaf growth stage (Gs16) (Zadoks *et al.* 1974), wheat plants were thinned to five plants per pot. During this time, all pots were watered daily and weighed to 75% of the field capacity for each individual soil using de-ionized water. After this period, soils in the pots were maintained at field capacity by frequent weighing and watering. Nitrogen fertiliser, as NH$_4$NO$_3$ (34% N) was applied at 214 mg/pot, every 10 days after emergence for seven applications and then applied weekly. A total of 4.5 g NH$_4$NO$_3$ was applied per pot from emergence to maturity.

There were two harvests. At the sixth leaf stage (Gs16), shoots of five plants were cut at ground level (harvest 1) and washed with DI water. The youngest emerged blades (YEB) were separated from the remainder of the shoots (Reuter *et al.* 1997b). Plant tissue samples were then oven-dried at 70°C for 48 hr and weighed. At maturity (early December), the five remaining plants were harvested at ground level (harvest 2) and separated into shoots and heads, oven-dried, and weighed. Grain was removed by hand-threshing to measure grain yields. The remaining husks were weighed and analysed with the rest of the straw to measure Zn concentration in shoots. Plant tissue from harvest 1 and 2 was analysed for Zn by atomic absorption spectrophotometry (see section 2.1.3).

**Analysis of Data:** All data were analysed by standard analysis of variance. In addition, simple linear regression and stepwise multiple regression were performed using standard statistical techniques outlined in analysis of data of Chapter 2.1.3.
Table 2.6. Soil properties, DTPA extractable Zn levels (mg/kg) of the soil with no Zn applied and the observed and predicted critical DTPA extractable levels of soils for wheat calculated from Equation 3.

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<th>Clay ^c</th>
<th>OC ^d</th>
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^a Soil numbers correspond to Table 2.1 and location in Appendix 1.
^b Soil pH measured in CaCl2 (Rayment and Higginson, 1992).
^c Clay content (Day, 1965).
^d Organic carbon content (Walkley and Black, 1934).
^e DTPA soil extractable Zn from the soil with no added Zn (as collected from the field).
^f Estimated critical DTPA Zn value for the soil from Cate and Nelson procedure (Cate and Nelson, 1967, 1985).
^g Estimated critical level of DTPA Zn calculated from the multiple linear regression (Equation 3).
^h NR, non-responsive soils to applied Zn. Soil 16, gave a 5 % yield increase to applied Zn.

The relationship between dry matter or grain yield (dependent variable, y axis), and the
concentration of Zn in YEB (independent variable, x axis), was used to define critical Zn concentrations in YEB. The critical value is the Zn concentration in YEB as outlined in analysis of data of Chapter 2.1.3. The Mitscherlich equation of Barrow and Mendoza (1990) was used:

\[ y = a - b \exp(-c x) \]  

(1)

where \( y \) is the yield of dried shoots or grain yield, \( x \) is the concentration of Zn in YEB and \( a, b, \) and \( c \) are coefficients. Coefficient \( a \) provides an estimate of the asymptote or maximum (relative) yield plateau. Coefficient \( b \) estimates the difference between the asymptote and the intercept on the yield axis at \( x = 0 \). Therefore, \( b \) indicates the maximum increase in yields (yield response) due to changes in the concentration of Zn in plant parts. Coefficient \( c \) describes the shape (curvature coefficient) of the relationship and governs the rate at which \( y \) (the yield response) increases as \( x \) increases. Mean data were fitted to the equation by non-linear regression using a computer program written in compiler BASIC (Barrow and Mendoza 1990). The simplex method (Nelder and Mead 1965) was used to locate the least squares estimate of the non-linear coefficients.

As discussed in the analysis of data of Chapter 2.1.3, hand fitted curves are often more appropriate than fitted equations. In this study and following chapters, the Mitscherlich and hand drawn curves were used to estimate critical Zn concentrations in plant parts. Often the fitted equation gave unrealistic \( c \) coefficients as the relationship between the concentration of Zn in plant parts and yield are very steep. Similarly, the estimated \( a \) coefficient (maximum yield) did not correspond to the data. However, fitted equations are reported where appropriate fits were made.

The critical \( \text{Zn}_{\text{DTPA}} \) value for the soil was calculated by the Cate-Nelson procedure as outlined in Chapter 2.1.3. Simple, stepwise multiple linear regressions were used to determine the relationships between critical \( \text{Zn}_{\text{DTPA}} \) and soil properties, and confidence
limits of the critical $Zn_{\text{DTPA}}$ were determined as outlined in Chapter 2.1.3.

2.2.4 Results

*Plant symptoms and responses to applied Zn:* Symptoms of Zn deficiency were observed early in the growth of seedlings: the extent of the symptoms depending upon the severity of the deficiency. A longitudinal pale green stripe on each side of the mid-rib of fully emerged leaves, a symptom of mild deficiency (Snowball and Robson 1983), was observed on several soil types. The leaf tissue within the stripe died and the necrotic area turned pale brown. On soils where the deficiency was more severe there was a general paling of the leaves. Where the deficiency was severe the wheat seedlings were stunted and the necrotic areas increased about half way along the leaves, causing them to collapse and droop.

![Graph showing the relationship between zinc concentration and relative shoot dry yield](image)

**Figure 2.8** The relationship between the percentage maximum dry weight of wheat shoots and the Zn concentration in the youngest fully emerged blade (YEB) sampled at Gs16. *Maximum dry weight of shoots for the 0.8 mg Zn/pot is equivalent to 100%.*

The addition of Zn increased the Zn concentration in plant parts (YEB, straw and grain) for wheat grown on all soils. Zinc application did not increase dry weight of shoots and
grain yield for six of the soils (Table 2.6). For the total dry matter (grain and straw) produced at maturity, Zn deficiency was associated with concentrations of Zn in the YEB of less than 11 mg/kg at harvest 1. There was a good relationship at harvest 1 between Zn concentration in YEB and the total dry weight of plants (Figure 2.8). A critical concentration of Zn in YEB of less than 11 mg/kg agrees with the findings of other researchers (Reuter et al. 1997a; Brennan et al. 1993).

**Soil-extractable Zn:** A good relationship between total dry yield and Zn\textsubscript{DTPA} from the soil was achieved. The critical Zn\textsubscript{DTPA} value is defined as the soil test value where 90% of the maximum yield of wheat straw and grain yield was achieved. For Zn responsive soils, the critical Zn\textsubscript{DTPA} values varied between 0.12 and 0.27 mg Zn/kg (Table 2.6). The critical Zn\textsubscript{DTPA} value was closely related ($R^2 = 0.64$) to the pH\textsubscript{Ca} of the soil (Figure 2.9a). The simple linear regression relationship between critical Zn\textsubscript{DTPA} value and the clay content (%) of the soil explained about 54% of the variation (Figure 2.9 b). However, for multiple regression a good relationship was obtained between three soil properties and the Zn\textsubscript{DTPA} value. The soil properties of soil pH\textsubscript{Ca}, organic carbon (OC) (%) content and clay (%) content were selected from a stepwise regression analysis based on the critical "t" value for each independent variable.

The model was:

$$ Y (Zn\textsubscript{DTPA}) = 0.041 + 0.019 pHCa + 0.003 clay (%) + 0.004 OC (%) $$  \hspace{1cm} (3)

In this equation, Y is the critical Zn\textsubscript{DTPA} value and has a coefficient of determination ($R^2$) of 0.878. The coefficients and standard errors of the model are listed in Table 2.7. The confidence limit for the range of critical Zn\textsubscript{DTPA} has been calculated as $\pm 0.012$ mg Zn/kg.

**Properties of responsive soils:** Table 2.6 presents the soil properties. Both the organic carbon and the clay content were generally low, resulting in most soils being in a narrow
Figure 2.9 The linear regression relationships between critical DTPA Zn and (a) soil pH\textsubscript{Ca}, (b) clay content (%) and (c) organic carbon content (%) of the responsive soils. All linear relationships are significant. The critical values of correlation coefficient ($R$) is 0.296 at $p = 0.05$.
soil textural class (sands). This class represents the majority of soils in WA where Zn needs to be applied (Gartrell and Glencross 1968; McArthur 1991). Only four of the 36 soils that were Zn-deficient were alkaline (pH > 7), and only one soil (No. 26) contained free calcium carbonate (0.7 %). The range and mean of the soil properties were: clay (1.5 to 27 %, 6.9 %), pH\textsubscript{Ca} (3.9 to 7.7, 5.3), organic carbon (0.39 to 4.7, 1.2 %), aluminium oxide (0.02 to 1.6 %, 0.22 %), and iron oxides (0.02 to 2.3 %, 0.63 %).

Table 2.7. Multiple linear regression model for estimating critical concentration of DTPA extractable soil Zn from several soil properties of soil deficient in Zn for maximal (grain and straw) growth of wheat (Equation [3]). Also listed are the standard error (SE), the t-statistic, the probability (Prob.), and the coefficient of determination (R\textsuperscript{2}) of the fitted equation.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficients</th>
<th>SE</th>
<th>t Statistic</th>
<th>Prob.</th>
<th>R\textsuperscript{2}</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH\textsubscript{Ca}</td>
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<td>0.002</td>
<td>7.703</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Clay</td>
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<td>0.0005</td>
<td>6.176</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Organic carbon</td>
<td>0.004</td>
<td>0.002</td>
<td>2.143</td>
<td>0.039</td>
<td>0.878</td>
</tr>
<tr>
<td>Intercept</td>
<td>0.041</td>
<td>0.169</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Soil extractable Zn for the non-responsive soils:** The critical Zn\textsubscript{DTPA} values for the non-responsive soils were calculated using Equation 3. Critical Zn\textsubscript{DTPA} values for soil numbers 11, 31, 37, 41, 43 and 44 (Table 2.6) were 0.14, 0.18, 0.21, 0.26, 0.22 and 0.21 mg Zn/kg soil, respectively. With the exception of soils 31 and 44, these estimated critical DTPA values were less than the levels of Zn\textsubscript{DTPA} (Table 2.6) measured for each of these soils when no Zn was applied. That is, the measured Zn\textsubscript{DTPA} of the soil with no applied Zn was greater than the estimated critical soil level determined by Equation 3. Therefore, no yield increase was expected, and this was so. For example, for soil 31 (Table 2.6), the calculated critical Zn\textsubscript{DTPA} was equal to the value measured by DTPA for the collected soil with no Zn application and there was no yield response to applied Zn. For soil 44, there was a 5 % yield increase to applied Zn fertiliser. The critical Zn\textsubscript{DTPA}, estimated by Equation 3, adequately identified both Zn responsive and non-responsive soil for wheat yield.
The critical soil test level of DTPA extractable Zn (mg Zn/kg) for maximum wheat growth varied from 0.12 to 0.27, depending upon soil type (Table 2.6). These DTPA extractable Zn (Zn$_{DTPA}$) values are much lower than the values derived from other studies where both the soil pH$_{Ca}$ and clay content was generally higher than soils of WA. For example, Zn$_{DTPA}$ values (mg Zn/kg) for soils of higher clay content and pH in India were 0.46 (Rathore et al. 1978) or 0.65 (Singh et al. 1980). Use of critical Zn$_{DTPA}$ values derived from such international work would result in excessive usage of Zn for sandy soils in WA.

Other workers (Haq and Miller 1972; Martens 1968) have correlated levels of various chemical extractants of soil Zn with soil properties. Some researchers (Bansal et al. 1980; Sillanpää 1982) have separated soils into Zn responsive and non-responsive classes using DTPA extractable Zn; these authors have not attempted to define the soil properties that determine the critical levels for each responsive soil. However, only a few studies (Chapter 2.1) have used either simple or multiple linear regressions to relate critical Zn$_{DTPA}$ values measured for individual soils with their properties, as has been done in the present research for the predominantly sandy soils found in WA.

The properties of the 36 responsive soils used to determine the multiple linear regression for wheat resulted in a narrower range of soil properties, particularly those properties that affect the availability of Zn, compared to the clover study (compare Table 2.1 to Table 2.6). In the clover study (Chapter 2.1) about 88 % of the variation in the critical Zn$_{DTPA}$ level was explained by the soil pH$_{Ca}$ value. In the wheat study there was a range of pH$_{Ca}$ values from about 4 to 8 and only 55 % of the variation in the critical Zn$_{DTPA}$ value was explained by the soil pH$_{Ca}$ value. In the wheat study about 60 % of the soils have a clay content of less than 15 %, so the sampled population of the soils was biased to the sandy
textural classes of soil. However, the soils of south west WA are very sandy and only a small proportion (<20 %) of the State has soils that are high in clay content (McArthur 1991). Therefore the soil Zn testing procedure based on the relationship between critical $Zn_{DTPA}$ and soil properties is relevant to sandy soils, the predominant soil type of WA. The data cannot be confidently extended to the neutral to alkaline loam and clay soils of the region, that comprise about 25 % of the 18 million ha used for agriculture in south-western WA. These soils have higher pH values, higher clay contents and sometimes higher free calcium carbonate contents, and further research is required to develop appropriate regression equations for these soils. Compared to the wheat study, the regression equation for soil test Zn developed for clover (Chapter 2.1) included a wider range of soils with varying properties; hence it may not have the same limitation as the Zn soil test calibrated for wheat. However, for both the clover and wheat studies, the same soil properties were selected by the regression analysis ($pH_{Ca}$, clay content) and for wheat (organic carbon).

**Practical implications:** A test that identifies soils that are responsive to Zn fertiliser for wheat production would help farmers to decide when they should apply Zn fertiliser. This study suggests that the critical $Zn_{DTPA}$ required for maximum wheat production can be determined for each soil if the soil $pH_{Ca}$, clay content (%) and organic carbon (%) are measured. The appropriate equation for either clover or wheat would then be employed.

Where clover and wheat were grown on the same soil types (compare Table 2.1. and Table 2.6.) it was observed that the critical DTPA soil test value for clover tended to be higher than the respective values for wheat (Fig 2.10).

For either clover or wheat, if the critical $Zn_{DTPA}$ value is higher than the measured soil DPTA-extractable Zn level, Zn fertiliser is recommended. Similarly, if the measured soil DTPA values were higher than the critical $Zn_{DTPA}$ from the fitted model, no Zn fertiliser would be recommended.
However, when the critical $Zn_{DTPA}$ value of a soil approaches the measured DPTA-extractable Zn level of a particular soil, the standard error about the model (either for clover or wheat) is used. The main purpose of using the upper confidence interval of the critical $Zn_{DTPA}$ would be to ensure that fertiliser Zn was applied to reach and maintain maximum grain yields of wheat and clover.

*Practical field calibrations:* For 30 field sites where Zn fertiliser has been applied to wheat (see experiments listed in Chapter 4) it was found that where the $Zn_{DTPA}$ of the nil Zn treatment was greater than the calculated critical $Zn_{DTPA}$, no increase in grain yield to Zn fertiliser was measured (Table 2.8).

Consequently, the soil test adequately identified non-responsive sites in the field. For site 30, the measured $Zn_{DTPA}$ of the soil was below the critical $Zn_{DTPA}$ and an increase in grain yield was measured (Table 2.8).
Table 2.8. Evaluation of the DTPA soil test Zn in experimental field sites where soil properties were measured and critical DTPA Zn soil test values were determined for grain yield increases of wheat. See Chapter 4 for details of experiments.

<table>
<thead>
<tr>
<th>Site</th>
<th>pH&lt;sub&gt;Ca&lt;/sub&gt;</th>
<th>Clay&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>OC&lt;sup&gt;b&lt;/sup&gt; (%)</th>
<th>DTPA Zn (mg/kg)</th>
<th>Grain yield&lt;sup&gt;e&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6.1</td>
<td>10</td>
<td>1.1</td>
<td>0.188</td>
<td>0.5 N</td>
</tr>
<tr>
<td>2</td>
<td>7.0</td>
<td>12</td>
<td>1.3</td>
<td>0.209</td>
<td>0.4 N</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
<td>12</td>
<td>1.4</td>
<td>0.201</td>
<td>0.4 N</td>
</tr>
<tr>
<td>4</td>
<td>6.1</td>
<td>13</td>
<td>1.6</td>
<td>0.203</td>
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</tr>
<tr>
<td>5</td>
<td>7.2</td>
<td>12</td>
<td>1.0</td>
<td>0.207</td>
<td>0.3 N</td>
</tr>
<tr>
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<td>5.1</td>
<td>8</td>
<td>1.2</td>
<td>0.170</td>
<td>0.5 N</td>
</tr>
<tr>
<td>7</td>
<td>5.2</td>
<td>9</td>
<td>0.7</td>
<td>0.172</td>
<td>0.3 N</td>
</tr>
<tr>
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<td>6</td>
<td>1.1</td>
<td>0.169</td>
<td>0.3 N</td>
</tr>
<tr>
<td>9</td>
<td>6.2</td>
<td>4</td>
<td>1.2</td>
<td>0.161</td>
<td>0.6 N</td>
</tr>
<tr>
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<td>6.1</td>
<td>3</td>
<td>1.2</td>
<td>0.166</td>
<td>0.4 N</td>
</tr>
<tr>
<td>11</td>
<td>5.4</td>
<td>5</td>
<td>1.4</td>
<td>0.163</td>
<td>0.6 N</td>
</tr>
<tr>
<td>12</td>
<td>5.3</td>
<td>7</td>
<td>1.6</td>
<td>0.168</td>
<td>0.3 N</td>
</tr>
<tr>
<td>13</td>
<td>6.1</td>
<td>8</td>
<td>1.1</td>
<td>0.173</td>
<td>0.4 N</td>
</tr>
<tr>
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<td>6.1</td>
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<td>0.6</td>
<td>0.167</td>
<td>0.4 N</td>
</tr>
<tr>
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<td>1.0</td>
<td>0.163</td>
<td>0.3 N</td>
</tr>
<tr>
<td>16</td>
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<td>0.7</td>
<td>0.163</td>
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</tr>
<tr>
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<td>1.2</td>
<td>0.173</td>
<td>0.2 N</td>
</tr>
<tr>
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<td>1.7</td>
<td>0.201</td>
<td>0.7 N</td>
</tr>
<tr>
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<td>6</td>
<td>0.9</td>
<td>0.151</td>
<td>0.8 N</td>
</tr>
<tr>
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<td>5</td>
<td>0.8</td>
<td>0.147</td>
<td>0.7 N</td>
</tr>
<tr>
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<td>3.5</td>
<td>0.7</td>
<td>0.148</td>
<td>0.4 N</td>
</tr>
<tr>
<td>22</td>
<td>5.6</td>
<td>4</td>
<td>1.5</td>
<td>0.151</td>
<td>0.4 N</td>
</tr>
<tr>
<td>23</td>
<td>5.5</td>
<td>2</td>
<td>0.8</td>
<td>0.152</td>
<td>0.3 N</td>
</tr>
<tr>
<td>24</td>
<td>5.4</td>
<td>4</td>
<td>1.0</td>
<td>0.149</td>
<td>0.3 N</td>
</tr>
<tr>
<td>25</td>
<td>5.4</td>
<td>3</td>
<td>0.7</td>
<td>0.147</td>
<td>0.5 N</td>
</tr>
<tr>
<td>26</td>
<td>5.3</td>
<td>2</td>
<td>1.2</td>
<td>0.144</td>
<td>0.3 N</td>
</tr>
<tr>
<td>27</td>
<td>5.7</td>
<td>4.5</td>
<td>1.0</td>
<td>0.160</td>
<td>0.3 N</td>
</tr>
<tr>
<td>28</td>
<td>6.3</td>
<td>2</td>
<td>1.2</td>
<td>0.161</td>
<td>0.6 N</td>
</tr>
<tr>
<td>29</td>
<td>6.2</td>
<td>3</td>
<td>0.8</td>
<td>0.164</td>
<td>0.4 N</td>
</tr>
<tr>
<td>30</td>
<td>5.7</td>
<td>3.5</td>
<td>0.7</td>
<td>0.156</td>
<td>0.1 Y</td>
</tr>
</tbody>
</table>

<sup>a</sup>Field sites from a range of experimental sites of the Department of Agriculture.

<sup>b</sup>pH<sub>Ca</sub>, clay content, organic carbon (OC) content measured on soil collected from plots where no Zn fertiliser had been applied.

<sup>c</sup>Critical DTPA Zn as calculated from Equation 3.

<sup>d</sup>DTPA soil extractable Zn measured at the field site where no Zn fertiliser had been applied.

<sup>e</sup>Y is where an increase in grain yield was measured; N is where no increase in grain yield was measured.

2.2.6 Conclusion

In this chapter, it was shown that when soil pH, clay, OC and CaCO₃ content of soils were considered, critical DTPA-Zn levels predicted the need for Zn fertiliser on a range of sandy soils for wheat and clover. Further field calibrations of the DTPA Zn soil test developed for other crop species are required for WA soils. Commercial laboratories now offer the DTPA soil test for Zn fertiliser recommendations in WA and other States of Australia (Armour and Brennan 1999).
Chapter 3

The Effectiveness of Zinc Fertilisers

3.1 Effectiveness of Zn for subterranean clover

3.1.1 Abstract

In the previous chapter it was shown that provided soil pH, clay, organic carbon (OC) and CaCO₃ content of soils were considered, critical soil Zn levels were accurate in predicting the need for Zn fertiliser on a wide range of sandy soils for wheat and clover. The effectiveness of an application of Zn for growth depends on a range of soil properties. However, the extent to which soil properties change the effectiveness of freshly applied Zn (initial effectiveness; IE) fertiliser has not been studied in WA. The extent of soil reactions between Zn fertiliser and soil properties was studied to determine the amount of Zn required to increase the availability of Zn for maximum dry matter yield of clover (cv. Nungarin) shoots for a wide range of Australian soils in a glasshouse experiment. The IE of Zn fertiliser was found to vary markedly among soil types when IE values were determined by measuring; (i) the dry weight of shoots (DWS), and (ii) Zn content (uptake) of clover. The IE of the Zn application for dry weight of shoots (IE_DWS) on a range of soils were found to be related to the pH_Ca and the level of DTPA soil extractable Zn (Zn_DTPA) measured in the unfertilised soil. The following stepwise linear regression model best described IE measured by dry weight of shoots (IE_DWS):

\[ Y (IE_{DWS}) = 26.54 - 1.187 \text{pH}_{Ca} - 48.52 \text{DTPA Zn}_0. \ (n = 45; R^2 = 0.86). \]

The IE based on Zn uptake (IE\textsubscript{uptake}) by clover shoots was:

\[ Y (IE\textsubscript{uptake}) = 103.712 - 10.062 \text{pH}_{Ca} - 12.752 \text{ZnDTPA} \ (n=54; \ R^2 = 0.85). \]

The IE of Zn fertiliser measured by DTPA soil extraction (IE\textsubscript{DTPA}) was also found to vary markedly among soil types. The level of Zn extracted by DTPA after the addition of Zn fertiliser was affected by clay content (%), organic carbon (OC) content (%) and calcium carbonate (CaCO\textsubscript{3}) (%) content of the range of Australian soils. This relationship could be described by:

\[ Y (IE\textsubscript{DTPA}) = 0.178 + 0.002 \text{clay} (%) + 0.014 \text{OC} (%) + 0.018 \text{CaCO}_3 (%) \ (n = 54; \ R^2 = 0.84). \]

Hence, extractability of Zn was explained by variation of clay, organic carbon and calcium carbonate content, the same factors that affected critical DTPA Zn for clover: uptake of Zn and plant dry matter were in turn explained by DTPA Zn in conjunction with organic carbon and soil \text{pH}\textsubscript{Ca}. These results extend those of Chapter 2 by showing that not only does accurate prediction of Zn deficiency depend on a consideration of soil pH, OC and CaCO\textsubscript{3}, but also the initial effectiveness of Zn fertiliser recommended for low Zn soils.

3.1.2 Introduction

The effectiveness of sources of Zn fertilisers has been studied in both field and glasshouse experiments (Mortvedt and Gilkes 1993). Various workers have studied the movement of Zn out of fertiliser granules to determine their availability to plants (Mortvedt and Giordano 1967; Hossner and Blanchar 1969; Giordano and Mortvedt 1969; Mortvedt and Giordano 1969a, b; Giordano \textit{et al.} 1971; Gilkes \textit{et al.} 1975; Gilkes 1977; Giordano and Mortvedt 1978; Gilkes and Sadlier 1981). Similarly, the reaction of Zn with the various carrier fertiliser compounds has been studied to determine their effectiveness for a range
of plant species (Jackson et al. 1962; Ellis et al. 1965; Allen and Terman 1966; Terman et al. 1966; Lehr 1972). However, few studies have examined the initial effectiveness (IE) of a Zn fertiliser mixed in the soil, and its relationship to soil properties that may influence the availability of the Zn source. Fertiliser Zn forms a range of reaction products in the soil through dissolution, chelation, sorption and precipitation reactions, which affect the availability of the Zn for plant uptake and dry matter production. The extent of these reactions between a soil and Zn depends on the physical and chemical properties of the soil. These reactions will determine the amount of Zn fertiliser required to overcome Zn deficiency for plant growth. The IE of Zn, as measured by a change in plant growth, content of Zn in plant parts, or a change in the extraction of Zn by DTPA has been poorly defined for soils of WA. This information is important to predict amounts of Zn fertiliser to be applied to achieve the desired levels of Zn availability in the soil.

The slope of the relationship between the amount of Zn applied and the change in either plant parameters (dry yield and content of Zn) or the change in extractable Zn$_{\text{DTPA}}$ is defined in this paper (following the approach used by Bolland et al. (1984) for P) as the IE of the applied Zn. The change in plant parameters and Zn$_{\text{DTPA}}$ indicates the extent to which Zn reacts with the soil. Martens et al. (1966) and Martens (1968) found that the amount of Zn extracted from the soil by DTPA varied with soils of varying soil properties. In this chapter, the IE values were related to measured soil properties in order to determine which soil properties control the availability of currently (freshly) applied Zn to plants.

3.1.3 Materials and Methods

A total of fifty four soils were collected; from WA (43 soils), South Australia (3 soils), Victoria (3 soils) and Queensland (5 soils). Soil properties (pH$_{\text{Ca}}$, clay content, organic
carbon (OC) content, iron (Fe₂O₃) and aluminium (Al₂O₃) oxides, and Zn (DTPA) were
determined by procedures described earlier (Chapter 2.1.3). The experimental design and
procedures for growth of clover have been outlined in a previous section (Chapter 2.1.3).

Table 3.1. The initial effectiveness (IE) of Zn applied to soils used in glasshouse experiments. Values for IE
for DWS and uptake are multiple by 100.

<table>
<thead>
<tr>
<th>No.</th>
<th>DWS⁺</th>
<th>Upt.⁺</th>
<th>DTPA⁺</th>
<th>No.</th>
<th>DWS</th>
<th>Upt.</th>
<th>DTPA</th>
</tr>
</thead>
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</tr>
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</tr>
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</tr>
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<td>5.54</td>
<td>0.16</td>
<td>40</td>
<td>21.2</td>
<td>6.72</td>
<td>0.19</td>
</tr>
<tr>
<td>14</td>
<td>NR</td>
<td>2.95</td>
<td>0.18</td>
<td>41</td>
<td>NR</td>
<td>3.96</td>
<td>0.19</td>
</tr>
<tr>
<td>15</td>
<td>19.0</td>
<td>5.13</td>
<td>0.15</td>
<td>42</td>
<td>NR</td>
<td>4.34</td>
<td>0.26</td>
</tr>
<tr>
<td>16</td>
<td>15.1</td>
<td>3.38</td>
<td>0.29</td>
<td>43</td>
<td>NR</td>
<td>4.34</td>
<td>0.20</td>
</tr>
<tr>
<td>17</td>
<td>17.7</td>
<td>5.24</td>
<td>0.21</td>
<td>44</td>
<td>12.6</td>
<td>3.00</td>
<td>0.30</td>
</tr>
<tr>
<td>18</td>
<td>NR</td>
<td>4.07</td>
<td>0.27</td>
<td>45</td>
<td>9.7</td>
<td>3.37</td>
<td>0.26</td>
</tr>
<tr>
<td>19</td>
<td>16.3</td>
<td>5.53</td>
<td>0.20</td>
<td>46</td>
<td>NR</td>
<td>5.20</td>
<td>0.20</td>
</tr>
<tr>
<td>20</td>
<td>17.8</td>
<td>4.28</td>
<td>0.21</td>
<td>47</td>
<td>10.9</td>
<td>2.84</td>
<td>0.30</td>
</tr>
<tr>
<td>21</td>
<td>13.8</td>
<td>4.21</td>
<td>0.22</td>
<td>48</td>
<td>9.0</td>
<td>2.27</td>
<td>0.33</td>
</tr>
<tr>
<td>22</td>
<td>16.8</td>
<td>3.37</td>
<td>0.25</td>
<td>49</td>
<td>5.4</td>
<td>3.50</td>
<td>0.31</td>
</tr>
<tr>
<td>23</td>
<td>9.0</td>
<td>2.70</td>
<td>0.33</td>
<td>50</td>
<td>11.3</td>
<td>2.66</td>
<td>0.36</td>
</tr>
<tr>
<td>24</td>
<td>15.7</td>
<td>3.83</td>
<td>0.22</td>
<td>51</td>
<td>8.8</td>
<td>3.23</td>
<td>0.34</td>
</tr>
<tr>
<td>25</td>
<td>14.1</td>
<td>3.89</td>
<td>0.21</td>
<td>52</td>
<td>7.8</td>
<td>3.12</td>
<td>0.34</td>
</tr>
<tr>
<td>26</td>
<td>17.0</td>
<td>4.67</td>
<td>0.20</td>
<td>53</td>
<td>NR</td>
<td>1.74</td>
<td>0.48</td>
</tr>
<tr>
<td>27</td>
<td>10.6</td>
<td>3.14</td>
<td>0.22</td>
<td>54</td>
<td>8.8</td>
<td>2.48</td>
<td>0.42</td>
</tr>
</tbody>
</table>

⁺Soil numbers correspond with those outlined in Chapter 2.1.3. Soil properties listed in Table 2.1.
⁺⁺Dry weight of shoots; g of plant shoots/μg of Zn applied.
⁺⁺⁺Zinc uptake; mg Zn in shoots/μg of Zn applied.
⁺⁺⁺⁺DPTA soil Zn; mg Zn extracted/μg Zn applied.
⁺⁺⁺⁺⁺NR. Non responsive soil, dry weight of shoots did not increase with Zn fertiliser.

Analysis of Data: The approach of Barrow and Campbell (1972) and Bolland et al. (1984)
was used. The IE of soil applied Zn was determined, based on either yield (IE_DWS), Zn
uptake (IE_uptake) or soil extractable Zn (IE_DTPA) from the slope (B) of the linear
relationship:

\[ Y = A + BX; \]  

(1)
where \( A \) is the dry matter of shoots or the Zn content of shoots or the level of DTPA extractable Zn where no Zn has been applied to the soil, and \( X \) is the amount of Zn added immediately before sowing the clover (currently applied Zn). All data were analysed statistically as outlined in Section 2.1.3.

### 3.1.4 Results

**Plant Growth and Zn Deficiency Symptoms:** The severity of symptoms of Zn deficiency and the dry matter response of clover to applied Zn depended on the soil type as outlined in Chapter 2.1.

**Initial Effectiveness of freshly applied Zn measured by dry weight and Zn content of clover shoots:** The initial effectiveness of freshly applied Zn in alleviating deficiency and hence increasing dry weight (IE\(_{\text{DWS}}\)) and Zn content of clover shoots (IE\(_{\text{uptake}}\)) varied markedly among soils (Table 3.1).

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Coefficient of determination (R(^2)) for:</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 46</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IE for DWS (IE(_{\text{DWS}}))</td>
<td>Clay</td>
<td>0.017 ns(^a)</td>
</tr>
<tr>
<td></td>
<td>( \text{A}_2\text{O}_3)</td>
<td>0.121 *</td>
</tr>
<tr>
<td></td>
<td>( \text{Fe}_2\text{O}_3)</td>
<td>0.010 ns</td>
</tr>
<tr>
<td></td>
<td>Organic C</td>
<td>0.106 *</td>
</tr>
<tr>
<td></td>
<td>( \text{CaCO}_3)</td>
<td>0.297*</td>
</tr>
<tr>
<td></td>
<td>( \text{pH}_{\text{Ca}})</td>
<td>0.479*</td>
</tr>
<tr>
<td></td>
<td>DTPA Zn</td>
<td>0.806*</td>
</tr>
<tr>
<td>IE for Zn content (IE(_{\text{uptake}}))</td>
<td>Clay</td>
<td>0.373 *</td>
</tr>
<tr>
<td>n = 54</td>
<td>Organic C</td>
<td>0.0008 ns</td>
</tr>
<tr>
<td></td>
<td>( \text{A}_2\text{O}_3)</td>
<td>0.007 ns</td>
</tr>
<tr>
<td></td>
<td>( \text{Fe}_2\text{O}_3)</td>
<td>0.017 ns</td>
</tr>
<tr>
<td></td>
<td>( \text{CaCO}_3)</td>
<td>0.396 *</td>
</tr>
<tr>
<td></td>
<td>DTPA Zn</td>
<td>0.226*</td>
</tr>
<tr>
<td></td>
<td>( \text{pH}_{\text{Ca}})</td>
<td>0.709*</td>
</tr>
</tbody>
</table>

\(^a\) Significant values (P<0.05); ns not significant.

\(^b\) The R\(^2\) value is for the multiple regressions involving the independent variable in that row and those in following rows.

For IE\(_{\text{DWS}}\), the level of Zn\(_{\text{DTPA}}\) in the collected soils with no added Zn explained 81 % of the variation (Table 3.2, Figure 3.1). For IE\(_{\text{DWS}}\), the soil \( \text{pH}_{\text{Ca}}\) value explained about 48 %
of the variation (Table 3.2, Figure 3.2). The simple linear relationship between \( I_{E_{DWS}} \) and the clay content of the soil explained about 48 % of the variation observed in \( I_{E_{DWS}} \) (Figure 3.3). Many of the correlations between \( I_{E_{DWS}} \) can soil properties are negative (Figure 3.1, 3.2, 3.3). None of the other soil properties were highly correlated with \( I_{E_{DWS}} \) in simple linear regressions. The inclusion of \( p_{H_{Ca}} \) with DTPA\( _{Zn} \) in a multiple linear regression improved the relationship and 86 % of the variation in \( I_{E_{DWS}} \) was explained. Adding other soil properties in the multiple linear regression did not further improve the ability to predict \( I_{E_{DWS}} \). The \( I_{E_{DWS}} \) (Table 3.4) could be described by:

\[
I_{E_{DWS}} = 26.548 - 1.187 \, p_{H_{Ca}} - 48.348 \, DTPA \, Zn_0. \tag{2}
\]

Table 3.3. The regression relationships between the initial effectiveness (IE) of applied Zn fertiliser measured by the level of DTPA extractable Zn (DTPA\( _{Zn} \)) and soil properties of Australia soils where (a) all factors were included and where (b) calcium carbonate was excluded.

<table>
<thead>
<tr>
<th>Depend. variable(^a)</th>
<th>Indep. variable(^b)</th>
<th>( R^2 ) Simple Regression</th>
<th>( R^2 ) Multiple Regression</th>
<th>( R^2 ) Simple Regression</th>
<th>( R^2 ) Multiple Regression</th>
</tr>
</thead>
<tbody>
<tr>
<td>( I_{E_{DWS}} ) of DTPA extractable Zn</td>
<td>DTPA( _{Zn} )</td>
<td>0.152 ( ^c )</td>
<td>0.854*</td>
<td>( Fe_2O_3 )</td>
<td>0.006 ns</td>
</tr>
<tr>
<td>( p_{H_{Ca}} )</td>
<td>0.475*</td>
<td>0.853*</td>
<td>Organic C</td>
<td>0.007 ns</td>
<td>0.701*</td>
</tr>
<tr>
<td>( A_12O_3 )</td>
<td>0.006 ns</td>
<td>0.853*</td>
<td>( A1_2O_3 )</td>
<td>0.006 ns</td>
<td>0.694*</td>
</tr>
<tr>
<td>(( n = 54 ))</td>
<td>( Fe_2O_3 )</td>
<td>0.006 ns</td>
<td>0.850*</td>
<td>DTPA( _{Zn} )</td>
<td>0.151*</td>
</tr>
<tr>
<td>Organic C</td>
<td>0.007 ns</td>
<td>0.838*</td>
<td>( p_{H_{Ca}} )</td>
<td>0.475*</td>
<td>0.687*</td>
</tr>
<tr>
<td>Clay</td>
<td>0.622*</td>
<td>0.795*</td>
<td>Clay</td>
<td>0.622*</td>
<td>0.673*</td>
</tr>
<tr>
<td>( CaCO_3 )</td>
<td>0.704*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( ^a \)Dependent variable.

\( ^b \)Independent variables of the stepwise multiple linear regression.

\( ^c \)Significant values at \( P<0.05 \); ns not significant.

The \( I_{E_{DWS}} \) and \( I_{E_{uptake}} \) values were generally lower on the alkaline (\( p_{H_{Ca}} > 7 \)) relative to the acidic (\( p_{H_{Ca}} < 7 \)) soils (Figure 3.2). However, there was still considerable variation in IE for both acidic and alkaline soils. The soil \( p_{H_{Ca}} \) alone explained about 71 % of the variation in \( I_{E_{uptake}} \) (Figure 3.2). Zn\( _{DTPA} \) only explained about 21 % of the variation in \( I_{E_{uptake}} \). None of the other soil properties were significantly correlated with \( I_{E_{uptake}} \) using simple linear regression. However, including both \( p_{H_{Ca}} \) and the level of Zn\( _{DTPA} \) for the nil
Zn treatment of the soil improved the relationship with 78 % of the variation explained in a multiple linear regression. The IE\textsubscript{uptake} (Table 3.4) was described by:

\[ IE_{uptake} = 103.718 - 10.063 \text{pH}_{Ca} - 12.754 \text{DTPAzn}_0. \]

Table 3.4. Multiple linear models for IE of fertiliser Zn, where IE was determined by dry weight of shoots (DWS) of clover (IE\textsubscript{DWS}), Zn content of clover shoots (IE\textsubscript{uptake}) and the DTPA extraction of soil applied Zn (IE\textsubscript{DTPA}).

<table>
<thead>
<tr>
<th>Variables\textsuperscript{a}</th>
<th>Coefficients</th>
<th>se.\textsuperscript{b}</th>
<th>t statistic\textsuperscript{c}</th>
</tr>
</thead>
<tbody>
<tr>
<td>IE\textsubscript{DWS}</td>
<td>pH\textsubscript{Ca}</td>
<td>-1.187</td>
<td>0.305</td>
</tr>
<tr>
<td>(n = 46)\textsuperscript{d}</td>
<td>DTPA Zn</td>
<td>-48.348</td>
<td>4.864</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>26.548</td>
<td>1.548</td>
</tr>
<tr>
<td>IE\textsubscript{uptake}</td>
<td>pH\textsubscript{Ca}</td>
<td>-10.063</td>
<td>0.893</td>
</tr>
<tr>
<td>(n = 54)</td>
<td>DTPA Zn</td>
<td>-12.754</td>
<td>3.178</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>103.718</td>
<td>5.043</td>
</tr>
<tr>
<td>IE\textsubscript{DTPA}</td>
<td>Clay</td>
<td>0.002</td>
<td>0.0004</td>
</tr>
<tr>
<td>(n = 54)</td>
<td>Organic carbon</td>
<td>0.014</td>
<td>0.0038</td>
</tr>
<tr>
<td></td>
<td>CaCO\textsubscript{3}</td>
<td>0.018</td>
<td>0.0028</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>0.178</td>
<td>0.0045</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Soil properties were independent of each other.
\textsuperscript{b} Standard error of coefficients.
\textsuperscript{c} Statistically significant values for coefficient at P < 0.001.
\textsuperscript{d} The number of soils analysed is shown in parentheses.

\textbf{DTPA soil extractable Zn:} The ability of DTPA to extract applied Zn varied twofold among soils (Table 3.1). The CaCO\textsubscript{3} content of the soil was the single soil property that best explained IE measured by Zn\textsubscript{DTPA} from the nil Zn treated soil (IE\textsubscript{DTPA}) values (Table 3.3). In a simple regression, the clay content of the soils explained 62 % of the variation in IE\textsubscript{DTPA}. The relationship between IE\textsubscript{DTPA} and soil pH\textsubscript{Ca}, explained about 47 % of the variation (Figure 3.4). The multiple linear regression between IE\textsubscript{DTPA} and organic carbon, clay content and free calcium carbonate (Table 3.4) explained 84 % of the variation with:

\[ IE_{DTPA} = 0.178 + 0.002 \text{clay} (%) + 0.014 \text{OC} (%) + 0.018 \text{CaCO}_3 (%) \]  \hspace{1cm} (4)

As only 7 soils in the set of soil used in this study contained free calcium carbonate, the
Figure 3.1 The relationship between the initial effectiveness of zinc measured by dry weight of shoots (IE \_DWS) (g of plant shoots/\( \mu \)g of Zn applied) and soil DTPA extractable zinc (mg/kg) for the nil zinc treatment.

Figure 3.2 The simple linear relationship between soil pH \_Ca and the initial effectiveness of zinc measured by dry weight of shoots (IE \_DWS) (g of plant shoots/\( \mu \)g of Zn applied) (∗) or by zinc content of shoots (IE \_uptake) (mg Zn in shoots/\( \mu \)g of Zn applied) (■).

Multiple linear regression was re-run after this soil property was excluded from the stepwise analysis. The \( R^2 \) decreased to 0.672, and the model was:
$IE_{DTPA} = 0.007 + 0.002\, pH_{Ca} + 0.0003\, Clay\, (%)$ \hspace{1cm} (5)

**Figure 3.3** The simple linear relationship between clay content (%) and the initial effectiveness of zinc measured by dry weight of shoots ($IE_{DWS}$) (g of plant shoots/μg of Zn applied) (♦), Zn content of shoots ($IE_{uptake}$) (mg Zn in shoots/μg of Zn applied) (■), and by DTPA extractable Zn ($IE_{DTPA}$) (mg Zn extracted/μg Zn applied) (▲).

**Figure 3.4** The relationship between soil pH$_{Ca}$ and the initial effectiveness of zinc measured by DTPA soil extractable Zn (mg Zn extracted/μg Zn applied) ($IE_{DTPA}$).
3.1.5 Discussion

The initial effectiveness (IE) of applied Zn for plant uptake and dry weight varied among soils and was generally negatively correlated with the soil properties, soil pH$_{Ca}$ and DTPA Zn in the soil, prior to Zn amendment. The IE$_{DTPA}$ was related to clay content and the soil pH$_{Ca}$. As the pH$_{Ca}$ increased IE declined suggesting the availability of Zn for uptake and growth of clover declined. The pH of the soil often determines the response of plants to Zn applications by decreasing the availability of both indigenous and applied Zn. However, curiously, a point requiring further work, the IE as measured by DTPA Zn extraction increased with pH$_{Ca}$. In general, the smaller the value of the DTPA Zn extracted in the unfertilised soil, the larger the dry matter response to the applied Zn.

Alkaline soils and those containing free calcium carbonate often require higher levels of Zn than acid soils to correct deficiencies in crops (Clark and Graham 1968; Navrot and Ravikovitch 1969; Lindsay 1972a; Saeed and Fox 1977). In alkaline calcareous soils there may be precipitation of Zn(OH)$_2$, ZnCO$_3$ or calcium zincate compounds of lower availability for plant uptake (Rogers and Wu 1948; Clark and Graham 1968; Saeed and Fox 1977; Mattigod et al. 1981). Udo et al. (1970) showed that the adsorption of Zn by carbonates results in Zn being less available for plant uptake. The solubility of Zn is highly dependent on pH, there being a 3 to 4 fold decrease in solubility of Zn with each unit increase in soil pH (Ravikovitch et al. 1968; Navrot and Ravikovitch 1969; Lindsay 1972a; Barber 1984; Moraghan and Mascagni 1991). The sorption of Zn on surfaces of clay and organic matter also increases as the soil pH increases (Jahiruddin et al. 1985, 1986).

Brennan (1992) found that the IE of Zn as measured by Zn content of dried clover shoots (IE$_{uptake}$) was less in soils with higher levels of organic carbon. However, in the stepwise multiple linear regression of the present study where pH$_{Ca}$ was used instead of pH in...
water, organic carbon was not selected by the regression analysis. However, clay, organic carbon and the calcium carbonate content of the soil affected the $I_{E_{DTPA}}$. Martens *et al.* (1966) found that an increase in organic matter at constant clay levels increased Zn bound by the organic clay complex which in turn increased the DTPA extractability of soil Zn.

The extractability of Zn by DTPA declined as the soil pH and clay content increased (John 1972, 1974; Havlin and Soltanpour 1984). However, in their work, the soils were sampled after corn plants were grown for eight weeks after planting, which would allow reactions of Zn with the soil and for Zn uptake by plants. The reactions of Zn with soil properties are important in decreasing the residual effectiveness of the application of Zn fertiliser (Chapter 2.1). In Chapter 2.1 for clover growth, the critical levels of $Zn_{DTPA}$ for soils were found to be positively related to both $pH_{Ca}$ and the clay content. This relationship between the critical $Zn_{DTPA}$ level and soil properties was statistically improved by the inclusion of free calcium carbonate. Similarly, for wheat growth (Chapter 2.2), the critical $Zn_{DTPA}$ level was positively related to $pH_{Ca}$, clay content and organic carbon. It would appear that the soil properties controlling Zn availability in a range of Australian soils are $pH_{Ca}$, clay content, organic carbon and free calcium carbonate, particularly at $pH_{Ca}$ above about 7.5.

### 3.1.6 Conclusions

The DTPA soil test for Zn was initially developed for alkaline calcareous soils with high clay contents (Lindsay and Norvell 1978). However, it has subsequently been used on a range of acid to neutral soils (Haynes and Swift 1983) and appeared to predict Zn responses for a range of species in that study.

The present study has determined that $pH_{Ca}$, clay content, organic carbon and free calcium carbonate reduce the effectiveness of freshly applied Zn on a range of Australian soils. These four soil properties continue to reduce the availability of Zn resulting in the need to
reapply Zn when soil Zn becomes deficient and crop production declines. Aspects of the RV of Zn fertiliser particularly at superphosphate applications less than 150 kg/ha, and increased usage of imported high analysis NP fertiliser low in Zn in WA are discussed in following Chapters.
3.2 Effectiveness of Zn foliar sprays for wheat

3.2.1 Abstract

When soil Zn is insufficient to prevent deficiency, there may be a need to apply a corrective Zn application during crop growth. Foliar sprays of Zn chelate (EDTA; 15 % Zn) and Zn sulfate (22.4 % Zn) are used to correct Zn deficiency when it appears in existing crops. The effectiveness of Zn chelate compared to the effectiveness of Zn sulfate (relative effectiveness) for the grain yield of wheat was determined. The experiments were conducted on Zn-deficient soils at 3 sites in 3 different years, in the Newdegate district about 350 km, south-east of Perth, WA. Each source was sprayed at 6 levels of Zn to 2 growth stages of wheat, to define the relationship between grain yield and the amount of Zn applied. The levels of Zn sprayed were 0, 25, 50, 100, 200 and 400 g Zn/ha for experiment 1; and 0, 28, 56, 112, 225 and 450 g Zn/ha for experiments 2 and 3. For comparison between soil applied and foliar in the timing of Zn, plots received adequate soil applied Zn while sowing the wheat seed.

Foliar applied Zn chelate was 1.4 to 1.7 times more effective than Zn sulfate when applied at the 4th leaf (Gs14) growth stage. However, the Zn chelate and Zn sulfate sprays were equally effective when applied at the 3 to 4 tiller (Gs23-24) growth stage. Delaying the foliar application of Zn from Gs14 until Gs24 of wheat resulted in a 20 % reduction in the maximum grain yield achieved at 2 experimental sites (Experiment 1 & 2). At another site (Experiment 3), delaying the foliar application of Zn from the Gs14 to Gs24 resulted in about a 30 % decrease in grain yield. However, Zn sulfate banded (drilled) with the seed at sowing produced the highest grain yield of wheat.

An earlier version was published as “Effectiveness of Zn sulfate and Zn chelate as foliar sprays in alleviating zinc deficiency of wheat grown on zinc deficient soils in Western Australia”. RF Brennan (1991), Aust. J. Exp. Agric. 31, 831-834.
For example, at experiment 1 the grain yield of wheat for the Zn drilled with the seed was 2.54 t/ha compared to the early Zn foliar spray that produced about 2.3 t/ha; about an 8% decrease for delaying the correction of Zn deficiency. Foliar Zn sprays can minimize yield loss when applied to crops that express Zn deficiency symptoms during growth. However, Zn supply needs to be adequate from sowing since even a short delay in the foliar supply of Zn decreased grain yield.

3.2.2 Introduction

With the decreasing use of Zn-enriched superphosphate in agriculture and increased usage of herbicides which can reduce root growth and Zn uptake by crops (Robson and Snowball 1989, 1990; Osborne and Robson 1992), the prevalence of Zn deficiency in crops can be expected to increase, necessitating better strategies for correcting Zn deficiency in crops. Zinc sulfate is widely used as a foliar spray in situations where Zn deficiency has been diagnosed in the growing crop (Duncan 1967; Murphy and Walsh 1972; Martens and Westermann 1991). Chelated Zn compounds were first used in agriculture about 30 years ago because they were considered more effective than inorganic Zn salts (Wallace and Wallace 1982). However, chelated Zn as a source of Zn to overcome Zn deficiency of wheat has never been evaluated for wheat growing in the wheatbelt of WA.

The experiments described in this section compare the effectiveness of chelated Zn (Zn-EDTA, 15% Zn) with Zn sulfate (ZnSO₄·7H₂O, 22.4% Zn) applied as foliar sprays to increase the grain yield of wheat grown on low Zn soil. Relative effectiveness (RE) values were determined by comparing the amount of Zn from each source required to produce the same grain yield.

3.2.3 Materials and Methods
General procedures: The experiments were conducted in 3 different years (1984-86) at 3 sites in the Newdegate district (mean annual rainfall 350 mm; with about 80 % of the rain falling in the May–October growing season), about 350 km south-east of Perth, WA (Table 3.5). The region has a Mediterranean climate with cool, wet winters (June–August) and warm to hot summers (December–February). All experiments were located on soil types that had been cleared of native vegetation in the previous year and had never received Zn fertiliser. Plots were 1.4 by 40 m and there was a 0.4 m strip of untreated soil between each plot. Buffer plots (1.4 by 40 m) were placed on either side of the plots receiving no foliar Zn, to minimise possible contamination of Zn from spray drift. All experiments were of randomised block design with 3 replicates. Soils at the experimental sites were classified according to Northcote (1979). Soil samples of the 0 to 10 cm profile were taken from 15 to 20 positions at random from each site before the experiments started. Soil properties (Table 3.5) were analysed by procedures outlined in Chapter 2.1.3.

Table 3.5. The soil properties of the experimental sites (0-10 cm) and the wheat cultivars sown.

<table>
<thead>
<tr>
<th>Wheat cultivar</th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th>Experiment 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil type</td>
<td>Yellow loamy sand (Uc 5.22)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Yellow earth (Uc 5.22)</td>
<td>Yellow gravelly sand (Gn 2.21)</td>
</tr>
<tr>
<td>pH&lt;sub&gt;Ca&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.1</td>
<td>5.2</td>
<td>5.2</td>
</tr>
<tr>
<td>Clay (%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>10</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>82</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td>Organic carbon (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.59</td>
<td>0.43</td>
<td>0.57</td>
</tr>
<tr>
<td>Zinc (mg/kg)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.16</td>
<td>0.15</td>
<td>0.12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Soil classification (Northcote 1979).
<sup>b</sup> Soil pH in 1:5 soil:0.01mol CaCl<sub>2</sub> (Rayment and Higginson 1992).
<sup>c</sup> Day (1965).
<sup>d</sup> Organic carbon (Walkley and Black 1934).
<sup>e</sup> DTPA extractable Zn (Lindsay and Norvell 1978).

For all experimental sites, basal fertilisers were applied (kg/ha), 125 DAP (18 % N, 20 % P), 150 gypsum (20 % Ca, 17 % S), 6 copper sulfate (25 % Cu) and 0.25 sodium molybdate (39 % Mo) were drilled with the seed. Further additions of urea (46 % N) at
50 kg/ha were applied to the soil surface 4 weeks after seeding. Soil test K (120 mg K/kg by bicarbonate extractable) indicated that the soil types were adequately supplied so no additions of K were made.

In each experimental design, two additional Zn treatments were applied. For one treatment, Zn fertiliser was drilled at 700 g Zn/ha; the second additional treatment received the drilled Zn fertiliser and an extra Zn foliar spray of 225 g Zn/ha, applied as Zn sulfate at Gs14. Twelve rows of seeds, 175 mm apart, were sown along each plot in mid-May. Seeds of the wheat cultivars (Table 3.5) were sown at 50 kg/ha at the 3 experiments. All weeds were successfully controlled using pre- and post-emergent herbicides. Grain was machine harvested from an area 1.1 by 40 m within each plot in early December of each year.

**Zinc spray treatments:** Zn sulfate and Zn chelate were sprayed on the plants in 100 L of water/ha. Each experiment had plots that received the Zn spray treatments at Gs14 and another set of plots that received the Zn spray treatments at Gs22-Gs24. Zinc was sprayed at 0, 25, 50, 100, 200 and 400 g Zn/ha for experiment 1, while for experiments 2 and 3 the levels were 0, 28, 56, 112, 225 and 450 g Zn/ha. Spraying was carried out on calm, cloudy days to minimise spray drift and increase the chances of uptake of Zn applied to the leaf surface (foliar uptake).

**Plant testing:** Thirty to forty youngest emerged leaf blades (YEB) were collected from the nil Zn spray treatments at Gs45. Samples of grain were collected by taking 40-50 mature heads before harvest to eliminate any possible chances of Zn contamination of the grain by machinery. The leaf and grain samples were digested and then analysed for Zn by atomic absorption spectrophotometry as outlined in Chapter 2.1.3.

**Analysis of data:** The relationship between grain yield and the amount of Zn applied was fitted by the Mitscherlich equation:
\[ Y = a - b \exp(-cx) \]  

where \( Y \) is the grain yield (t/ha); \( x \) is the level of Zn sprayed (g/ha), \( a \) is the maximum grain yield (t/ha); \( b \) is \((a-a_o)\) where \( a_o \) is the grain yield when no Zn spray had been applied, and \( c \) is the curvature coefficient. Within each experiment, the same \( A \) value was reached for both sources of Zn. The Mitscherlich model was fitted by a computer program using non-linear least squares with a modification of the Levensberg-Marquardt algorithm (Miller 1981).

The effectiveness of Zn chelate and Zn sulfate for correcting Zn deficiency in wheat was compared by the curvature coefficient (\( c \)) of the response curve and the amount of Zn required for 90 % of maximum yield. For grain yield response, this was done by dividing \( c \) of Zn chelate and Zn sulfate by \( c \) for the Zn sulfate. Therefore, by definition, the RE of the Zn sulfate was 1.0. Comparisons were made relative to sulfate because it is the major Zn source for WA and because the chelate was being investigated as a possible alternative source of foliar Zn spray for wheat crops. Secondly, the amount of Zn applied (g/ha) as a foliar application for 90 % of the maximum grain yield was calculated as 0.9 of the \( a \) value determined in Equation 6. The relative effectiveness of the sources of foliar applied Zn were calculated by dividing the amount of Zn required for 90 % of the maximum grain yield of Zn chelate and Zn sulfate by the amount Zn required for 90 % of the maximum yield for the sulfate source (RE\(_{90\%}\)). Therefore, by definition the RE\(_{90\%}\) for sulfate is equal to 1.0. If RE\(_{90\%}\) for Zn chelate is <1.0, the Zn chelate is less effective than Zn sulfate for producing grain yield so more Zn chelate needs to be applied as a foliar spray to produce 90 % of the maximum grain yield.

3.2.4 Results

**Plant symptoms of Zn deficiency**: Where no Zn had been sprayed, Zn deficiency symptoms were observed: affected plants were stunted; with a general paling of leaves
Figure 3.5 The effect of sources of zinc foliar spray on grain yields of wheat grown on Zn-deficient soils at site 1 (a and b), site 2 (c and d) and site 3 (e and f). The earlier Zn spray was applied at Gs14 (a, c, e); the later treatment at Gs22-24 (b, d, f). Symbols (♦) Zn chelate, (▲) Zn sulfate. Curves represent the fitted Mitscherlich model to the data. Fitted equations are shown in Table 3.6. Vertical bars represent standard errors of means. NB: Scale of y-axis is varied for clarity.
and necrotic areas about half way along the leaves, causing them to bend in the middle. The stunted plants often had a “water soaked” or “diesel soaked” appearance particularly in early growth stages. The plants with the “diesel soaked appearance” were severely Zn-deficient and often died or failed to produce grain.

Plants grown at the second highest level of Zn spray (200 or 225 g Zn/ha) had no symptoms of Zn deficiency. However, a single spray of Zn applied at 100 or 112 g Zn/ha at Gs22-24 failed to eliminate symptoms of Zn deficiency of wheat at all sites and for both times of foliar spray application. For these plots, there was a longitudinal pale green stripe on both sides of the mid-vein of YEB. The leaf tissue in the stripe died and the necrotic area turned pale brown.

Table 3.6. The effect of sources of Zn foliar spray on grain yield and relative effectiveness (RE) of Zn spray sources for grain yield of wheat grown on soils deficient in Zn (Experiments 1-3). The terms a, b, and c are parameters of the fitted Mitscherlich models (Equation 6) from Figure 3.5; $R^2$ is the coefficients of determination.

<table>
<thead>
<tr>
<th>Zinc source</th>
<th>a (t/ha) $(a \pm se)$</th>
<th>b (t/ha) $(a-a_o \pm se)$</th>
<th>c $(10^2 \times C)$</th>
<th>$R^2$</th>
<th>RE$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expt. 1, sprayed at Gs14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelate</td>
<td>2.30 ± 0.02</td>
<td>1.31 ± 0.02</td>
<td>1.05</td>
<td>0.94</td>
<td>1.48</td>
</tr>
<tr>
<td>Sulfate</td>
<td>2.32 ± 0.02</td>
<td>1.32 ± 0.02</td>
<td>0.71</td>
<td>0.91</td>
<td>1.00</td>
</tr>
<tr>
<td>Expt. 1, sprayed at Gs22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelate</td>
<td>1.97 ± 0.03</td>
<td>0.94 ± 0.05</td>
<td>2.36</td>
<td>0.96</td>
<td>1.00</td>
</tr>
<tr>
<td>Sulfate</td>
<td>2.01 ± 0.02</td>
<td>1.00 ± 0.04</td>
<td>2.37</td>
<td>0.92</td>
<td>1.00</td>
</tr>
<tr>
<td>Expt. 2, sprayed at Gs14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelate</td>
<td>1.81 ± 0.02</td>
<td>1.34 ± 0.06</td>
<td>1.07</td>
<td>0.97</td>
<td>1.57</td>
</tr>
<tr>
<td>Sulfate</td>
<td>1.76 ±0.03</td>
<td>1.27 ± 0.03</td>
<td>0.68</td>
<td>0.94</td>
<td>1.00</td>
</tr>
<tr>
<td>Expt. 2, sprayed at Gs23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelate</td>
<td>1.46 ± 0.02</td>
<td>0.95 ± 0.06</td>
<td>2.13</td>
<td>0.96</td>
<td>1.04</td>
</tr>
<tr>
<td>Sulfate</td>
<td>1.48 ±± 0.01</td>
<td>0.98 ± 0.03</td>
<td>2.04</td>
<td>0.93</td>
<td>1.00</td>
</tr>
<tr>
<td>Expt. 3, sprayed at Gs14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelate</td>
<td>2.11 ± 0.03</td>
<td>1.21 ± 0.03</td>
<td>0.79</td>
<td>0.98</td>
<td>1.52</td>
</tr>
<tr>
<td>Sulfate</td>
<td>2.13 ± ± 0.05</td>
<td>1.25 ± 0.05</td>
<td>0.51</td>
<td>0.93</td>
<td>1.00</td>
</tr>
<tr>
<td>Expt. 3, sprayed at Gs24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chelate</td>
<td>1.44 ± 0.01</td>
<td>0.54 ± 0.02</td>
<td>2.34</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>Sulfate</td>
<td>1.44 ± 0.01</td>
<td>0.54 ± 0.02</td>
<td>2.45</td>
<td>0.96</td>
<td>1.00</td>
</tr>
</tbody>
</table>

$^a$Relative effectiveness (RE) is the ratio of the curvature coefficient (c) for the chelate source divided by the c for the sulfate source. That is, by definition the RE for the sulfate is 1.00.
At the highest Zn level for either source of Zn, there was little damage to the foliage of the plants. This was so for both sources of foliar Zn sprayed at either growth stage of wheat.

**Grain yield:** Zinc applied as fertiliser at seeding gave the highest yields (P<0.05) and the additional Zn sulfate spray at Gs14 did not increase grain yield further. The grain yields with Zn applied with drilled fertiliser (and with Zn fertiliser plus the additional Zn spray in brackets) were 2.53 (2.54) t/ha for experiment 1, 1.90 (1.92) t/ha for experiment 2 and 2.25 (2.28) t/ha for experiment 3. Only 1 level of soil-applied Zn (recommended level for the soil type; Gartrell and Glencross 1968) was drilled with the seed.

There were large grain yield responses to foliar Zn sprays for all experiments (Figure 3.5). Grain yield of wheat was reduced where Zn deficiency was not corrected from early growth of wheat using Zn drilled with seed. The maximum yield was also higher when the Zn deficiency of wheat plants was treated by foliar Zn at the earlier growth stage (see *a* values in Table 3.6) rather than the later stage. For example in experiment 3, grain yield was 2.1 t/ha when sprayed at the highest amount of Zn at Gs14 growth stage while the same Zn spray treatment only yielded 1.4 t/ha when sprayed at Gs24 (Table 3.6). That is, about 0.7 t wheat grain/ha (about 30 % decrease in yield) was lost by delaying correction of Zn from the 4th leaf (Gs14) until early tillering (Gs24) (Table 3.6).

The amount of Zn required (g/ha) as a foliar spray to reach 90 % of the maximum grain yield (90 % of the *a* values of Table 3.6) varied with the source of Zn, time of application and the year the experiment was done (Table 3.7). For example, for foliar applied Zn at Gs14, the amount of Zn for 90 % maximum grain yield was 274 g/ha as the chelate and about 327 g Zn/ha as the sulfate source (Expt. 3; Table 3.7). At Gs24, both sources were identical in RE (Table 3.7) and the amount of Zn for 90 % of the maximum yield (g/ha) was about 66 for experiment 1, 85 for experiment 2 and 56 for experiment 3 (Table 3.7). That is, the amount of Zn required for 90 % of the maximum grain yield for the later
growth stage was about one-third of that required for the earlier growth stage of cereal.

**Table 3.7.** The amount of Zn (g/ha) applied as a foliar spray to achieve 90 % of the maximum yield for chelate and sulfate Zn applied at Gs14 and Gs24. Also listed is the relative effectiveness (RE) of the two sources of Zn. By definition RE for sulfate is 1.0.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Growth stage</th>
<th>Zn source</th>
<th>Zn$_{90}^a$ (g/ha)</th>
<th>RE$_{90}^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Gs14</td>
<td>chelate</td>
<td>172</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Gs14</td>
<td>sulfate</td>
<td>260</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>Gs24</td>
<td>Combined$^c$</td>
<td>66</td>
<td>nd$^d$</td>
</tr>
<tr>
<td>2</td>
<td>Gs14</td>
<td>chelate</td>
<td>187</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Gs14</td>
<td>sulfate</td>
<td>284</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Gs24</td>
<td>combined</td>
<td>85</td>
<td>nd$^d$</td>
</tr>
<tr>
<td>3</td>
<td>Gs14</td>
<td>chelate</td>
<td>274</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>Gs14</td>
<td>sulfate</td>
<td>327</td>
<td>1.00</td>
</tr>
<tr>
<td></td>
<td>Gs24</td>
<td>combined</td>
<td>56</td>
<td>nd$^d$</td>
</tr>
</tbody>
</table>

$^a$ The amount of Zn (g/ha) required for 90 % of maximum yield as determined from the Mitcherlich equation (see Table 3.6).

$^b$ The relative effectiveness for 90 % of maximum yield (RE$_{90}$) is defined as the amount of Zn required for the chelate source divided by the amount of Zn required for the sulfate source. Therefore by definition RE$_{90}$ for sulfate source is 1.00.

$^c$ Both sources of Zn as foliar spray resulted in identical response curves. That is the individual curves gave the same a and c coefficients, hence a combined response curve was fitted to experiment 1.

$^d$ The RE$_{90}$ could not be determined by the ratio.

**Plant concentration of Zn:** In YEB from the nil Zn spray plots collected at Gs45, Zn concentrations were <10 mg Zn/kg indicating that the wheat plants were deficient in Zn (Reuter and Robinson 1997; see also Chapter 2.2). The Zn concentration in the grain collected from the nil Zn spray plots were also <10 mg/kg, which would normally be associated with Zn deficiency of wheat.

**Effectiveness of the Zn sources:** For the Zn spray treatment applied at the earlier growth stage of wheat, Zn chelate consistently had a larger value for $c$ than the sulfate source, indicating the chelate was always more effective than Zn sulfate (Table 3.6). For example in experiment 1, the $c$ was 1.05 for the chelate source of Zn compared to 0.71 for the sulfate source. However, at the second time of spraying (Gs22 to Gs24), the 2 sources were about equally effective. For example, the $c$ of the two sources at Gs22 for experiment 1 was about 0.236 (Table 3.6).
As determined using grain yield, the RE of Zn chelate was about 1.5 (Table 3.6); that is, the chelate source of Zn was about 50% more effective for producing grain than Zn sulfate when sprayed at Gs14. Consequently, the amount of Zn as chelate to produce the same grain yield about 66% that of Zn sulfate (Table 3.7). The RE values for Zn chelate spray for the earlier time of spraying were similar for each experiment (Tables 3.6, 3.7).

As the sprays were equally effective at the second growth stage, a single relationship between grain yield of wheat and the amount of Zn applied as a foliar spray was determined (Figure 3.5). For the spray applied at the later time, for each source of Zn in each experiment, the Mitscherlich equations fitted to data for the relationship between grain yield and the amount of Zn applied were not significantly different.

3.2.5 Discussion

Zinc chelate is more effective than Zn sulfate as a foliar spray for alleviating Zn deficiency in wheat if applied to young plants before mid-tillering growth stage (Gs24). By mid-tillering, both sources were equally effective as foliar sprays in alleviating Zn deficiency of wheat. The reasons for this change in the effectiveness of the Zn chelate with time of spraying are not fully understood. The change in effectiveness of the chelate from Gs14 to Gs24 is unlikely to be due to weather conditions under which the sprays were applied as the conditions were selected to be similar (cool and cloudy). The effectiveness of a foliar application of Zn to correct Zn deficiency is a function of absorption of Zn at the leaf surface and the mobility of Zn within the leaf and plant tissue (Bukovac and Wittwer 1957; Bowen 1969; Beauchamp and Lean 1973) and these factors may have changed from Gs14 to Gs24.

The need for early application of Zn sprays to overcome Zn deficiency in the present work agrees with previous findings that Zn deficiency has to be corrected early (Duncan 1967; Brennan 1986; Sharma and Katyal 1986; Brennan 1998). For example, Duncan
(1967) found that Zn sulfate applied as a 1 % spray at 2 to 4 weeks after emergence of the seedlings alleviated symptoms of Zn deficiency and increased grain yields. However, applications of foliar Zn sprays 5 weeks after emergence were unable to alleviate the Zn deficiency. However, Samboornaraman et al. (1968) found that Zn foliar sprays of 1.12 kg Zn/ha at tillering (Gs20) were most effective for wheat grown in Zn deficient soils of India. MacNaeidhe and Fleming (1988) only sprayed Zn at Gs34 and found it to be effective in alleviating Zn deficiency to achieve maximum grain yield but there were no Zn sprays at earlier growth stages. That is, comparisons of the effectiveness of early applied and later applied sprays of foliar Zn were not made. No other data from the literature are available from which effectiveness and relative effectiveness of foliar Zn sources as outlined in this chapter can be made.

The levels of Zn sulfate spray required to increase grain yield to near maximum yields was within the range 1 to 2 kg ZnSO₄/ha (1-2 % solutions), which is the recommended amount of foliar Zn sulfate in WA (Brennan 1986, 1998, 2000). The work of MacNaeidhe and Fleming (1988) suggested that 10 kg ZnSO₄/ha as a foliar spray was toxic and reduced yields. Although the symptoms of Zn deficiency were severe in the present work, the amount of Zn as a foliar spray for maximum grain yield is similar to that found in the literature. For example, Takkar and Walker (1993) reported that foliar sprays of 0.5–1.0 kg ZnSO₄/ha (0.5 to 1.0 % Zn sulfate solutions) were required for maximum yields.

The amount of Zn required for maximum grain yield as a foliar spray declined from the early to later growth stage of wheat. The higher levels of Zn as a foliar application required at Gs14 compared to that required at Gs24 could be due to a large proportion of the early application falling to the soil surface. The roots of plants could access the Zn that falls on the soil surface provided the soil surface remains moist. Little of the surface applied Zn would move into the soil as Zn is firmly held by organic carbon, sesquioxides
and clay in the soil. The decrease in the amount of Zn product that was required for maximum yield from the early to later growth stage might also be due to the increase in leaf area to intercept the Zn spray. Leaf interception of the spray and the proportion of the Zn spray falling onto the soil surface were not measured in this study or in any other reported study in the literature. High levels of Zn that were toxic were not investigated and no toxic symptoms were observed at levels of Zn application used in this study. Dilute solutions of Zn sulfate (1 to 2 % Zn solutions) have been found elsewhere to be effective in eliminating Zn deficiency in plants (Chapman 1966; Duncan 1967; Murphy and Walsh 1972).

In this study, the drilled Zn fertiliser was more effective than a foliar spray applied either at an early growth stage or at mid-tillering of wheat. An additional Zn spray applied at an early growth stage to supplement the Zn fertiliser which was drilled with the seed did not further increase grain yield of wheat, suggesting that the Zn fertiliser was adequate in this work. To compare the effectiveness of soil applied Zn and foliar sources of Zn several amounts of each source would need to be applied to obtain yield response curves for both the solid fertiliser and the foliar spray. Soil application of Zn fertiliser banded with the seed resulted in the highest grain yield, and although the foliar Zn was sprayed early in the vegetative stage, there were grain yield losses (about 10 %) associated with early Zn deficiency (this study). Sharma and Katyal (1986) found that a soil application was more effective than a single foliar spray of 2.5 kg ZnSO₄/ha when the symptoms were observed at an early stage of growth, but Brown and Krantz (1966) demonstrated that banding Zn fertiliser reduced its availability to plants. In this study, however, the soil applied Zn was placed (drilled) with the sown wheat seed, which is the recommended procedure for applying fertiliser Zn to soil in WA.

The results of this work contrast with MacNaeidhe and Fleming (1988) who reported that a foliar application of Zn EDTA gave higher yields than Zn sulfate when sprayed at Gs34.
However, MacNaeidhe and Fleming (1988) used only two levels of Zn (compared to 6 levels of this study) and that is insufficient to define the relative effectiveness of the two sources. Although the effectiveness of Zn chelate was greater than that for Zn sulfate at Gs14, similar quantities of each product were required to achieve the same wheat grain yield because each source had a different Zn concentration. For example, 225 g Zn in the chelate form (1.50 kg of product) was as effective as 338 g Zn in the sulfate form (1.50 kg product). At later spraying times (Gs22-24), the same amount of Zn was required from each source; therefore, more chelate product was required for maximum yields. For example about 1.0 kg Zn sulfate/ha and 1.5 kg of Zn chelate/ha was required for maximum yield (see Experiment 1, Figure 3.5).

Foliar application of Zn is an emergency procedure where Zn deficiency of cereals has been observed (visual symptoms) in a standing crop or diagnosed by plant analysis. Since Zn sulfate sprays are inexpensive and give satisfactory results (often similar in effectiveness as other soluble Zn sources), the more expensive Zn chelates are probably unnecessary in cereal growing districts of WA. The need for early correction of Zn deficiency highlights the need for a soil test for Zn and a decision support system that will predict the need for Zn fertiliser before deficiencies of Zn are observed or measured in tissues of growing crops. The critical Zn$_{DTPA}$ values reported in Chapter 2 for clover and wheat can be adjusted for soils varying in pH$_{Ca}$, clay and calcium carbonate content and should serve the purpose of identifying low Zn soils before planting so that fertiliser Zn can be applied to the soil if required. The larger grain yield of wheat for the Zn fertiliser drilled with the seed at sowing compared to foliar Zn treatments suggest that early correction of Zn deficiency is essential. Therefore, soil Zn applications are recommended in WA.
3.2.6 Conclusions

The present study has determined those soil properties which reduce the effectiveness of freshly applied Zn. These soil properties continue to reduce the availability of Zn throughout the incubation of Zn with the soil. These continuing reactions of Zn with the soil could result in the need to reapply Zn if the Zn contaminant in superphosphate declines as other rock phosphate sources are used or applications of superphosphate decline to less than 150 kg/ha on many Western Australian soils. However, the length of time that Zn fertilizer remains effective for the production of maximum grain yield of wheat or dry matter production of clover is required for the soils of WA. The residual value of Zn for grain production of a range of crop species is examined in the following sections.
Chapter 4

Residual Value of Zinc to Field Crops.

4.1 Residual value of Zn using single superphosphate for wheat

4.1.1 Abstract

When first cleared for agriculture, most WA soils were too acutely deficient in Zn and phosphorus (P) for profitable wheat production, the major crop in the region. On farms, Zn was applied at 0.2 to 1.2 kg Zn/ha as ZnO when sowing the first crop, and ZnO was re-applied infrequently thereafter. The infrequent applications of Zn were a result of poor knowledge of the length of time that Zn remains effective for crop and pasture production. The length of time Zn fertiliser remains effective is called the residual value (RV). Farmers require an accurate knowledge of the RV of Zn fertiliser applied to soils of WA. However, the use of P fertiliser at >20kg P/ha, as single superphosphate contaminated with 400 to 600 mg Zn/kg, when applied annually masked any decline in the RV of Zn. Eventually as soil extractable P values increased, P was applied as superphosphate at <150 kg/ha single superphosphate when sowing crops. The RV of Zn was not known under these P fertiliser applications. Therefore, thirty long-term field experiments were done to assess whether the original Zn, as ZnO applied at a range of amounts of Zn (0.2 – 1.2 kg Zn/ha) up to 24 years earlier, still provided sufficient Zn for maximum grain production of wheat. This was achieved by applying fresh applications of ZnO to plots not previously treated with ZnO, but treated with Zn-contaminated superphosphate applied to all plots of the experiment each year.

An earlier version of this chapter was published as “Availability of previous and current applications of zinc fertiliser using single superphosphate for the grain production of wheat on soils of South Western Australia”. RF Brennan (1996), J. Plant Nutr. 19, 1099-1115.
Five levels of nitrogen were applied in all experiments to test if use of the N increased the requirement for Zn and reduced the residual value of the original ZnO treatments. At all sites, the current application of Zn fertiliser to soils previously treated up to 15 years previously with 0.2 to 1.2 kg Zn/ha as ZnO did not increase grain yield. The lowest level of Zn (0.2 kg Zn/ha, Experiment 17) applied 15 years earlier was still fully effective for maximum grain yield of wheat. The application of currently applied Zn increased the Zn concentration in the youngest fully expanded wheat leaves (blades) (YEB) in 23 experiments. Application of N decreased Zn concentration in YEB in 19 experiments, had no effect on the Zn concentration in 9 experiments, and increased Zn concentrations in two experiments, but did not induce Zn deficiency in any experiment. Evidently the original ZnO application, together with regular application of Zn-contaminated superphosphate, maintained adequate Zn in soil for grain production of wheat for at least 24 years.

4.1.2 Introduction

Wheat is the major crop grown on the predominately sandy neutral to acidic soils in WA. These soils comprise about 75 % of the approximate 18 million hectares used for broadacre agriculture in WA. Zinc oxide is the usual source of Zn applied to the soils of WA (Toms 1958; Gartrell and Glencross 1968).

Compared with applications to the soil of 0.60 to 2.6 kg Zn/ha, losses of Zn through leaching are negligible (Chapter 4) and removal of Zn in grain is small (Mengel and Kirby 1978; see also Chapter 7 for a more detailed analysis of Zn mass balances). As additions of Zn are high relative to that needed and removed in agricultural products it has been suggested that Zn should have a long residual value in the soils of WA. It has been demonstrated in glasshouse studies (Chapter 2) that when Zn fertiliser is mixed evenly through the soil in pots, the residual effectiveness of Zn declines with time from
application as a result of soil reactions. However, an adequate Zn supply for the grain yield of cereals may be maintained where at least 150 kg/ha of single superphosphate (9.1 % P, 10 % S, 20 % Ca, henceforth superphosphate) is applied annually, because Zn is a contaminant in superphosphate (400 to 600 mg Zn/kg) made from the Nauru and Christmas Island rock phosphates (Bingham 1959; Williams 1974). However, applications of superphosphate are now frequently declining to less than 150 kg/ha annually, and rock phosphate which has lower concentrations of Zn as impurities is being used more widely to manufacture superphosphate (Bolland 1999). Zinc deficiency is now being observed in many crops in WA. The increased use of DAP fertiliser, which contains about one-twelfth of the Zn found in superphosphate (Ghosh 1990; Mortvedt and Gilkes 1993), has often resulted in immediate Zn deficiency in crops (Brennan and Gartrell 1981).

High levels of N fertiliser supplied to plants grown in soil with marginal Zn supply has been found to induce Zn deficiency in those plants (Loneragan and Webb 1993). High levels of N applied to soils of WA have greatly increased grain yields of wheat (Mason and Rowland 1990; Mason and Brennan 2000) increasing the removal of Zn in grain. The increased usage of N fertilisers in WA may require higher and more frequent applications of Zn fertiliser for profitable wheat grain production than in the past. Hence in the context of significant changes in N and P fertiliser regimes, farmers require accurate information on the length of time that Zn applications remain fully effective in order to supply the Zn required for optimum grain production. Coupled with a reliable soil analysis method using DTPA as an extractant and a regression model that adjusts critical Zn levels according to pH_Ca, clay and calcium carbonate levels in the soil (Chapter 2), information on residual effects of Zn should enable farmers to avoid Zn deficiency.

This section assesses whether original applications of Zn (applied to the soil up to 24 years ago) are still sufficient for wheat crops to produce maximum grain yield. The field
experiments compared the original applications together with regular Zn-contaminated superphosphate to the effectiveness of currently applied Zn fertiliser. Several levels of N fertiliser were applied in combination with the original and current applications of Zn fertiliser to examine the possibility that high amounts of N fertiliser may increase the Zn demand in wheat rotations.

4.1.3 Materials and Methods

Soils and sites: There were 30 field experiments on soils in the WA wheatbelt that were Zn-deficient for cereal growth when first cleared of the original native plant vegetation and had never been treated with Zn fertiliser. Some properties of the soils, measured on soil samples before the experiments began using procedures outlined in Chapter 2.1.3, are listed in Table 4.1. Eleven experiments were in the Lake Grace-Newdegate district (mean annual rainfall 350 mm) about 300-350 km south-east of Perth; 10 experiments were in the Jerramungup district (mean annual rainfall 400-450 mm), 360 km south-east of Perth and nine experiments were in the Esperance district, (mean annual rainfall 490 mm) 600 km south-east of Perth.

Soils in the Lake Grace-Newdegate district were yellow-brown, lateritic, sandy earths (Gn 2.21, Northcote 1979), except in Experiment 9 (Tables 4.1, 4.2) which was located on a yellow earth (Ug 5.22). Soils in the Jerramungup district were duplex soils (sand over clay or sand over gravel) (Experiments 13, 14, 17, 20) (Dy 5.43) (Table 4.3). All the sites in the Esperance district (Table 4.4) were gravelly sands over clay (Dy 5.82). When the experiments first started, 9 to 24 years previously (1964-70) Zn was applied as ZnO at each site and amounts of these initial applications of Zn varied from 0.2 to 1.2 kg Zn/ha (Tables 4.2 to 4.4).

All trial sites were in pasture for 3 to 4 years before the experiments began, except Experiments 24, 28 and 29 which were sown to narrow-leaved lupin (Lupinus
*augustifolius* L.) the previous year. No Zn fertiliser was applied to these sites before the start of the original experiments, except for the Zn contamination present in earlier applications of single superphosphate (400 to 600 mg Zn/kg). A total of about 500 to 650 kg Zn-contaminated superphosphate (containing about 500 mg Zn/kg) had been applied to all sites before the experiments started, which would have supplied a total of between about 0.250 to 0.325 kg Zn/ha to soil before the experiments started.

*Experimental procedures:* The current experimental design was a randomised complete block with three replications sited on an area of known Zn fertiliser history. There was 0.4 m of untreated soil between each 1.4 by 40-m plot. Zinc fertiliser, as powdered Zn oxide (ZnO) (70 % Zn) that was freshly applied that year (currently applied Zn) was mixed with superphosphate (500 mg Zn/kg) which was then drilled at 200 kg/ha with the wheat seed sown at 50 kg/ha. There were four levels of N applied as urea (46 % N) in all experiments except 1, 2, 10, 11, 20, 21 and 23 which had three levels of applied N. The amounts of N applied were 0, 23, 46, 70 and 92 kg N/ha; not all these treatments were applied in all experiments (see Tables 4.2, 4.3, & 4.4). For example, there was no nil-N treatment for Experiments 3, 4, 5, 6 and the 70 and 92 kg N/ha treatments were not applied in Experiment 1, 2, 10 and 11 (see Table 4.2). The N fertiliser was applied to the soil surface before seeding in the drier districts (Lake Grace, Newdegate) and to the soil surface 4 to 5 weeks after seeding in the medium to higher rainfall areas (Jerramungup, Esperance), the recommended methods of applying N fertiliser to wheat in these areas (Mason 1979). Fertilisers other than N, drilled with the seed at all sites while sowing the first wheat crop at the start of each experiment, were CuSO$_4$.5H$_2$O (6.0 kg/ha) and NaMoO$_4$.2H$_2$O (0.25 kg/ha). In the higher rainfall districts (Jerramungup and Esperance), KCl (100 kg/ha: 50 % K) and gypsum (250 kg/ha, 17 % S) were applied to the soil surface 4 to 5 weeks after emergence.

Each year, paraquat (1.25 g/L) and diquat (75 g/L) plus surfactant were sprayed across all...
experimental sites in mid-May, and 1 week later Roundup (360 g glyphosate/L) was sprayed at 1.5 L/ha to control all weeds before seeding. Post-emergent weed control with 1.5 L Hoegrass /ha (375 g diclofopmethyl/L) controlled annual ryegrass (*Lolium rigidum* Gaudin) at all sites.

**Measurements:** Wheat seedlings at the 2-3 leaf stage (Gs12-13; Zadoks *et al.* 1974), 21 to 28 days after seeding, were counted along 1 m lengths of two adjacent rows at five random positions within each plot. During Gs50-59, to monitor Zn status of plants the youngest emerged blade (YEB) was sampled from 30 to 50 random positions in each plot. To measure Zn concentration in grain, 20 to 25 heads were collected by hand at random locations within each plot to minimise Zn contamination from machinery. Replicate samples of YEB and grain were ground and digested in a nitric-perchloric acid mixture and the Zn concentration in the digest was analysed by atomic absorption spectrophotometry (Allen 1961). Grain was measured by machine harvesting all grain within the middle 1.1 by 40 m section of each plot in late November-early December each year and the harvested grain (at moisture content 11%) was weighed.

**Soil-extractable zinc:** Soil samples from the 0 to 10 cm depth were collected from each plot not treated with freshly applied Zn. In addition, soil samples were collected from an adjacent uncleared area of virgin soil to which no fertiliser had ever been applied. The soil samples were collected in cores 10 cm long by 2.5-cm diameter. Thirty random soil samples were collected from each area or plot and bulked. The soil samples were air-dried, sieved (less than 2-mm fraction was used), and analysed for DTPA-extractable Zn (*Zn*$_{DTPA}$).

**Statistical analyses:** Analysis of variance was carried out and least significant differences (l.s.d) between treatment means within each experiment were calculated by reference to the t-distribution (Probability, P = 0.05).
4.1.4 Results

**Soil properties:** Table 4.1 lists some of the soil properties that were measured on soil samples collected from the 30 experiments before the current experiments began. The soil pH$_{Ca}$ values ranged from 4.7 to 6.7 (median 5.1), clay content ranged from 2 to 13 % (median 5 %) and organic carbon (OC) content ranged from 0.6 to 1.7 % (median 0.8 %). Zn$_{DTPA}$ ranged from 0.2 to 0.85 mg Zn/kg, and critical Zn$_{DTPA}$ estimated using the multiple regression model of Chapter 2 ranged from 0.17 to 0.27 mg Zn/kg (Table 4.1).

Table 4.1. The properties at the experimental sites in WA to measure wheat grain yield responses where Zn fertiliser had been previously applied. Also listed are the calculated critical DTPA Zn levels.

<table>
<thead>
<tr>
<th>Expt No</th>
<th>pH$_{Ca}$</th>
<th>Clay (%)</th>
<th>OC (%)</th>
<th>DTPA Zn (mg/kg)</th>
<th>Expt No</th>
<th>pH$_{Ca}$</th>
<th>Clay (%)</th>
<th>OC (%)</th>
<th>DTPA Zn (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>1.1</td>
<td>0.45</td>
<td>16</td>
<td>5.2</td>
<td>7.0</td>
<td>0.70</td>
<td>0.30</td>
</tr>
<tr>
<td>2</td>
<td>6.7</td>
<td>12.0</td>
<td>1.30</td>
<td>0.38</td>
<td>17</td>
<td>5.5</td>
<td>8.0</td>
<td>1.20</td>
<td>0.20</td>
</tr>
<tr>
<td>3</td>
<td>6.3</td>
<td>12.0</td>
<td>1.40</td>
<td>0.50</td>
<td>18</td>
<td>6.2</td>
<td>12.0</td>
<td>1.74</td>
<td>0.20</td>
</tr>
<tr>
<td>4</td>
<td>6.2</td>
<td>13.0</td>
<td>1.60</td>
<td>0.55</td>
<td>19</td>
<td>4.7</td>
<td>6.0</td>
<td>0.87</td>
<td>0.65</td>
</tr>
<tr>
<td>5</td>
<td>6.7</td>
<td>12.0</td>
<td>1.00</td>
<td>0.38</td>
<td>20</td>
<td>4.7</td>
<td>5.0</td>
<td>0.79</td>
<td>0.80</td>
</tr>
<tr>
<td>6</td>
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<td>8.0</td>
<td>1.20</td>
<td>0.52</td>
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<td>3.5</td>
<td>0.72</td>
<td>0.71</td>
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<tr>
<td>7</td>
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<td>9.0</td>
<td>0.70</td>
<td>0.35</td>
<td>22</td>
<td>4.9</td>
<td>4.0</td>
<td>1.47</td>
<td>0.38</td>
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<tr>
<td>8</td>
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<td>6.0</td>
<td>1.05</td>
<td>0.40</td>
<td>23</td>
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<tr>
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<td>5.5</td>
<td>4.0</td>
<td>1.20</td>
<td>0.85</td>
<td>24</td>
<td>4.9</td>
<td>4.0</td>
<td>0.97</td>
<td>0.32</td>
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<tr>
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<td>5.9</td>
<td>3.0</td>
<td>1.20</td>
<td>0.30</td>
<td>25</td>
<td>5.0</td>
<td>3.0</td>
<td>0.70</td>
<td>0.31</td>
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<tr>
<td>11</td>
<td>5.4</td>
<td>5.0</td>
<td>1.40</td>
<td>0.35</td>
<td>26</td>
<td>4.9</td>
<td>2.0</td>
<td>1.20</td>
<td>0.48</td>
</tr>
<tr>
<td>12</td>
<td>5.3</td>
<td>7.0</td>
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<td>0.58</td>
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<td>4.5</td>
<td>0.97</td>
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<td>8.0</td>
<td>1.10</td>
<td>0.30</td>
<td>28</td>
<td>5.8</td>
<td>2.0</td>
<td>1.15</td>
<td>0.30</td>
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<tr>
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<td>5.9</td>
<td>4.0</td>
<td>0.64</td>
<td>0.41</td>
<td>29</td>
<td>5.9</td>
<td>3.0</td>
<td>0.79</td>
<td>0.55</td>
</tr>
<tr>
<td>15</td>
<td>5.5</td>
<td>5.0</td>
<td>0.97</td>
<td>0.35</td>
<td>30</td>
<td>5.4</td>
<td>3.5</td>
<td>0.65</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*a Experiment number is the soil for the experimental sites listed in Table 4.2 for the Lake Grace-Newdegate district, Table 4.3 for the Jerramungup district and Table 4.4 for the Esperance district.

*b The calculated critical DTPA Zn soil test value from Chapter 2: Critical DTPA Zn = 0.04 + 0.019 pH + 0.003 clay (%) + 0.004 organic carbon (%).

**Plant growth and zinc deficiency symptoms:** Zn and N fertiliser treatments did not affect (P>0.05) the emergence of plants which varied from 110 to 130 plants/m$^2$. The numbers of emerged plants in all experiments were considered adequate for maximum wheat grain production (Anderson et al. 2000). Symptoms of Zn deficiency, either in the vegetative growth or at maturity, were not observed in any experiment.
Wheat Grain Yield: (a) Lake Grace-Newdegate district: Table 4.2 shows grain yield increase (response) to Zn fertiliser and grain concentration of Zn for a range of soils where Zn was applied in previous years. At all sites, currently applied Zn fertiliser did not increase yields. The initial application of Zn fertiliser at Sites 1, 2 and 11 which was applied 22 years before the currently applied Zn was still adequate for maximum wheat grain yield. Grain yields of wheat increased [l.s.d. = 0.1] with the application of N fertiliser at all sites (Table 4.2). Maximum wheat yields ranged from 1.4 to 2.4 t/ha and were comparable to the district averages for Lake Grace-Newdegate.

(b) Jerramungup: Table 4.3 shows grain yield and grain concentration response to Zn fertiliser for a range of soils where Zn was applied in previous years. Applied N fertiliser increased grain yield [l.s.d. = 0.13] but the addition of currently applied Zn fertiliser did not increase yields (Table 4.3). Zinc applied in 1964 at site 16 was still fully effective, 24 years after initial application (Table 4.3). Similarly, the application of Zn 23 years before the currently applied Zn at sites 13 and 19 was still adequate for maximum grain yield. Even where low levels of Zn had been applied 15 and 17 years (Experiments 17 and 18) previously, the current applications of Zn did not increased grain yields, and the high levels of N fertiliser did not induce Zn deficiency or reduce grain yield. Grain yields ranged from 1.2 to 2.9 t/ha and were comparable to district averages for Jerramungup.
Table 4.2. *Lake Grace-Newdegate district.* Effect of N on wheat grain yield (t/ha), and on Zn concentration in either the youngest emerged blade (YEB) or grain for Zn currently applied or applied 16-22 years previously. NL is the lowest level of applied N and NH the highest level for each experiment.

<table>
<thead>
<tr>
<th>Original, current year and level of applied Zn</th>
<th>DTPA Zn Treated</th>
<th>Zn concentration (mg/kg)</th>
<th>Grain Yield (t/ha) at N rate (kg/ha)</th>
<th>YEB</th>
<th>Grain</th>
</tr>
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<tbody>
<tr>
<td>1964*, 1986</td>
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<td>0.5, 0*</td>
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<td>0.14</td>
<td>1.1</td>
<td>1.3</td>
<td>1.4</td>
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<td>0.5, 0.6</td>
<td>1.0</td>
<td>1.3</td>
<td>1.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.6, 0</td>
<td>0.38</td>
<td>0.10</td>
<td>1.1</td>
<td>1.3</td>
<td>1.5</td>
</tr>
<tr>
<td>0.6, 0.6</td>
<td>1.2</td>
<td>1.4</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
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<tr>
<td>1970, 1986</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7, 0</td>
<td>0.50</td>
<td>0.10</td>
<td>-</td>
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<td>1.4</td>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1970, 1986</td>
<td></td>
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<tr>
<td>0.8, 0</td>
<td>0.55</td>
<td>0.12</td>
<td>-</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>0.8, 0.6</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>1967, 1987</td>
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<tr>
<td>0.6, 0</td>
<td>0.38</td>
<td>0.11</td>
<td>-</td>
<td>1.3</td>
<td>1.4</td>
</tr>
<tr>
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<tr>
<td>0.8, 0</td>
<td>0.52</td>
<td>0.12</td>
<td>-</td>
<td>1.4</td>
<td>1.5</td>
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<td>0.8, 0.6</td>
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<td>1968, 1987</td>
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<td>0.5, 0</td>
<td>0.35</td>
<td>0.13</td>
<td>0.7</td>
<td>1.2</td>
<td>1.4</td>
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<td>1.4</td>
<td>-</td>
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<td>0.11</td>
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<td>0.14</td>
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</tr>
<tr>
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<td>1.8</td>
<td>1.9</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>1967, 1986</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5, 0</td>
<td>0.30</td>
<td>0.15</td>
<td>1.8</td>
<td>2.2</td>
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</tr>
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<td>2.4</td>
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<td>1964, 1986</td>
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<td>0.13</td>
<td>1.2</td>
<td>1.4</td>
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<td>1.3</td>
<td>1.4</td>
<td>13</td>
<td>15</td>
</tr>
</tbody>
</table>

*a* The year of the original Zn application.

*b* The year of the current Zn application.

*c* The amount of the original, or subsequent Zn application (kg Zn/ha).

*d* Sample taken from original treated plots just before the current crop was sown.

*e* Sample taken from adjacent uncleared land that had never been fertilised.

*f* This N level was not applied in this experiment.
Table 4.3. Jerramungup district. Effects of N on wheat grain yield (t/ha), and on Zn concentration in either the youngest emerged blade (YEB) or grain for Zn currently applied or applied 8-24 years previously. *NL is the lowest level of applied N and NH the highest level for each experiment.*

<table>
<thead>
<tr>
<th>Applied Zn</th>
<th>DTPA Zn (mg/kg)</th>
<th>Grain Yield (t/ha) at N rate (kg/ha)</th>
<th>Zn concentration (mg/kg) YEB</th>
<th>Grain</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.6, 0°</td>
<td>Treated d, Unclear e</td>
<td>0.58 0.17 2.3 2.5 2.9 7.7</td>
<td>19 16 12 10</td>
<td></td>
</tr>
<tr>
<td>0.6, 0.7</td>
<td></td>
<td>2.4 2.6 2.9 -</td>
<td>19 17 12 12</td>
<td></td>
</tr>
<tr>
<td>0.6, 0°</td>
<td>0.30 0.13</td>
<td>2.1 2.5 2.6 -</td>
<td>13 10 13 13</td>
<td></td>
</tr>
<tr>
<td>0.6, 0.7</td>
<td></td>
<td>2.0 2.5 2.6 -</td>
<td>15 14 13 11</td>
<td></td>
</tr>
<tr>
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<td>0.41 0.12</td>
<td>2.2 2.9 2.9 -</td>
<td>15 10 14 12</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>2.1 2.9 3.0 -</td>
<td>21 20 21 18</td>
<td></td>
</tr>
<tr>
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<td></td>
</tr>
<tr>
<td>0.6, 0.7</td>
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<td>1.4 1.8 2.0 -</td>
<td>20 17 16 15</td>
<td></td>
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<tr>
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<td>0.8 1.1 1.4 -</td>
<td>13 10 13 10</td>
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</tr>
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<td>15 12 12 11</td>
<td></td>
</tr>
<tr>
<td>0.6, 0°</td>
<td>0.20 0.10</td>
<td>0.2 0.9 2.9 -</td>
<td>12 9 16 12</td>
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</tr>
<tr>
<td>0.6, 0.7</td>
<td></td>
<td>0.9 2.9 3.0 -</td>
<td>20 15 16 15</td>
<td></td>
</tr>
<tr>
<td>0.3, 0°</td>
<td>0.20 &lt;0.10</td>
<td>2.9 3.0 3.0 - 3.1</td>
<td>17 16 17 14</td>
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</tr>
<tr>
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<td>2.9 2.9 3.0 - 2.9</td>
<td>19 20 19 19</td>
<td></td>
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<tr>
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<td>0.65 0.12</td>
<td>2.4 2.5 2.7 - 2.8</td>
<td>12 12 16 13</td>
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<tr>
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<td>2.3 2.5 2.7 - 2.7</td>
<td>18 17 17 16</td>
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</tr>
<tr>
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<td>15 15 26 22</td>
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</tr>
<tr>
<td>1.2, 0.7</td>
<td></td>
<td>1.1 1.4 1.9 -</td>
<td>15 16 27 25</td>
<td></td>
</tr>
<tr>
<td>1.2, 0°</td>
<td>0.71 0.11</td>
<td>2.0 2.8 2.8 -</td>
<td>16 12 15 14</td>
<td></td>
</tr>
<tr>
<td>1.2, 0.7</td>
<td></td>
<td>2.1 2.9 2.9 -</td>
<td>16 16 19 14</td>
<td></td>
</tr>
</tbody>
</table>

Footnotes as for Table 4.2

(c) Esperance: Table 4.4 shows grain yield and grain Zn concentration increase to Zn fertiliser for a range of soils where Zn was applied in previous years. Currently applied Zn did not increase yield at any site (Table 4.4). At Experiments 27 and 28, Zn that had been applied 23 years previously was still sufficient for maximum yields (Table 4.4). The
addition of N fertiliser increased yields \( [\text{l.s.d.} = 0.1] \) at all sites irrespective of the previous Zn application except Experiment 24 where there was no response to N fertiliser (Table 4.4). Nitrogen fertiliser applied at the highest level did not reduce grain yield for any level of previously applied Zn. Grain yields ranged from 1.0 to 3.2 t/ha and were comparable to district averages for Esperance.

**Table 4.4. Esperance district.** Effect of N on wheat grain yields (t/ha), and on Zn concentration in either the youngest emerged blade (YEB) or grain for Zn currently applied or applied 8-24 years previously. *NL is the lowest level of applied N and NH the highest level for each experiment.*

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Original, current and level of applied Zn</th>
<th>Grain Yield (t/ha) at N rate (kg/ha)</th>
<th>Zn concentration (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DTPA Zn (mg/kg)</td>
<td>0</td>
<td>23</td>
</tr>
<tr>
<td>Experiment 22</td>
<td>Treated$^d$</td>
<td>0.6, 0$^c$</td>
<td>0.38</td>
</tr>
<tr>
<td>1964, 1986</td>
<td></td>
<td>0.6, 0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Experiment 23</td>
<td>0.6, 0</td>
<td>0.7, 0</td>
<td>0</td>
</tr>
<tr>
<td>1966, 1987</td>
<td></td>
<td>0.7, 0.7</td>
<td>1.1</td>
</tr>
<tr>
<td>Experiment 24</td>
<td>0.6, 0</td>
<td>0.7, 0</td>
<td>0</td>
</tr>
<tr>
<td>1967, 1988</td>
<td></td>
<td>0.6, 0.7</td>
<td>2.5</td>
</tr>
<tr>
<td>Experiment 25</td>
<td>0.5, 0</td>
<td>0.7, 0.7</td>
<td>0.5, 0</td>
</tr>
<tr>
<td>1966, 1987</td>
<td></td>
<td>0.5, 0</td>
<td>1.0</td>
</tr>
<tr>
<td>Experiment 26</td>
<td>0.7, 0</td>
<td>0.7, 0.7</td>
<td>0</td>
</tr>
<tr>
<td>1966, 1987</td>
<td></td>
<td>0.7, 0.7</td>
<td>0</td>
</tr>
<tr>
<td>Experiment 27</td>
<td>0.6, 0</td>
<td>0.7, 0</td>
<td>0.5, 0</td>
</tr>
<tr>
<td>1965, 1988</td>
<td></td>
<td>0.6, 0.7</td>
<td>1.0</td>
</tr>
<tr>
<td>Experiment 28</td>
<td>0.6, 0</td>
<td>0.7, 0</td>
<td>0.5, 0</td>
</tr>
<tr>
<td>1965, 1988</td>
<td></td>
<td>0.5, 0</td>
<td>0</td>
</tr>
<tr>
<td>Experiment 29</td>
<td>0.7, 0</td>
<td>0.7, 0</td>
<td>0.5, 0</td>
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<tr>
<td>1967, 1986</td>
<td></td>
<td>0.6, 0.7</td>
<td>0</td>
</tr>
<tr>
<td>Experiment 30</td>
<td>0.6, 0</td>
<td>0.7, 0</td>
<td>0.6, 0</td>
</tr>
<tr>
<td>1966, 1987</td>
<td></td>
<td>0.7, 0.7</td>
<td>0</td>
</tr>
</tbody>
</table>

Footnotes as for Table 4.2
Zinc concentrations in YEB at Gs50-59:

(a) Lake Grace-Newdegate district: Zinc concentrations in the YEB were >12 mg/kg dry weight, except in Experiment 8 when high levels of N and no Zn were applied (Table 4.2). In Experiment 8, application of N fertiliser decreased Zn concentration to 9 mg/kg where Zn was not currently applied. Zinc concentrations in the YEB increased with current applications of Zn in all experiments except with low N supply in Experiments 10 and 11 (Table 4.2). With currently applied Zn, as the level of N applied increased, Zn concentrations in the YEB generally decreased [l.s.d. = 0.8]. However, in Experiments 3, 9, 10, and 11 where Zn was applied in the current year, N fertilisers did not decrease the Zn concentration in YEB. Nitrogen application increased the Zn concentration in the YEB of wheat grown with previously and currently applied Zn on plots in Experiments 7 and 10.

(b) Jerramungup: Applying Zn fertiliser increased (l.s.d. = 1.25] the Zn concentration in the YEB in all experiments except Experiments 12, 20, and 21 for the lowest level of N applied (Table 4.3). For either previously or currently applied Zn, application of N decreased [l.s.d. = 1.25] the Zn concentrations, in the YEB, except on plots receiving a current application of Zn fertiliser in Experiments 18, 20, and 21 or on plots where no further Zn had been applied in Experiments 19 and 20. The Zn concentrations in the YEB where N was not applied were >12 mg/kg. Applying N fertiliser decreased the Zn concentration in the YEB to 10 mg/kg in Experiments 13, 14, 16, and 17 when Zn was not currently applied; <10 mg Zn/kg would be considered as the critical level in the YEB for wheat (Brennan et al. 1993; Weir and Cresswell 1994; Reuter et al. 1997a). Experiment 17 had the lowest level of previously applied Zn, an application of 0.2 kg Zn/kg 15 years before the current application. In Experiment 16, currently applied Zn at the highest level of N fertiliser resulted in a Zn concentration of 12 mg/kg in YEB.

(c) Esperance: Zinc concentration in the YEB was ≥10 mg/kg at all sites. Currently
applied Zn increased \([l.s.d. = 1.0]\) Zn concentrations in the YEB at all sites except 22 and 25 (Table 4.4). Applying N fertiliser decreased \([l.s.d. = 1.2]\) Zn concentration in the YEB except in Experiments 22, 25, 28, and 29 on plots with currently applied Zn. In Experiments 22, 24, 27, and 28, the concentration of Zn in the YEB decreased to \(<12 \text{ mg/kg}\) where N fertiliser but no current Zn fertiliser was applied. Experiment 24, which had a low amount of Zn fertiliser (0.6 kg/ha) applied 21 years before the currently applied Zn, had the lowest YEB concentration of Zn where no N and no current Zn was applied.

**Zinc concentrations in the grain:** In most experiments, Zn concentrations in the grain were above 10 mg/kg. However, where the highest level of N and no current Zn were applied, concentration of Zn in the grain declined to 10 mg/kg in some experiments [Experiments 1, 11 (Table 4.2); Experiments 12, 16 (Table 4.3); Experiments 22, 24, and 28 (Table 4.4)]. Currently applied Zn increased the concentrations of Zn in the grain except in Experiments 11, 12, 13, 16, 17, 21, 22, 29, and 30 (Tables 4.2 to 4.4).

Adding N fertiliser decreased the concentration of Zn in the grain for both the previously and currently applied Zn in about 70 % of the experiments (Tables 4.2 to 4.4). However, if Zn was not applied, N had no effect on Zn concentration in the grain for Experiments 7, 9, 10 (Table 4.2), Experiment 13 (Table 4.3), and Experiment 30 (Table 4.4). There were also some cases where Zn was currently applied and N fertiliser had no effect on the concentration of Zn in the grain [Experiments 10, 12, 18, 25, 19, and 30 (Tables 4.2 to 4.4)].

**Soil-extractable zinc:** Levels of Zn$_{DTPA}$ for the experimental sites were generally \(\geq 0.3 \text{ mg Zn/kg}\) in the previously Zn treated soils, except for Experiments 17 and 18 which were 0.2 mg Zn/kg. These levels of Zn$_{DTPA}$ were adequate for grain yield. The Zn$_{DTPA}$ from the plots with previously applied Zn reflected the levels of Zn that had been applied. The levels of Zn$_{DTPA}$ measured in soil samples taken from adjacent areas of unfertilised soil
were considerably lower than the previously fertilised experimental sites (Tables 4.2, 4.3, 4.4).

4.1.5 Discussion

Generally, the re-applications of Zn to soil previously treated with Zn fertiliser did not increase wheat grain yields in these experiments. Periods up to 23 years had elapsed since the last Zn application. Even with a low level of Zn (0.2 kg/ha) applied 15 years previously, the soil Zn supply was still adequate for maximum grain yield at the site. This suggests that the original Zn applications, and regular applications of Zn-contaminated superphosphate, especially where >150 kg/ha were applied to the experimental plots, maintained the soil Zn status for grain production of wheat. The time before Zn deficiency is observed would depend on the initial amount of Zn applied, how long since it was applied and the history of superphosphate applications to legume crops or pastures between the cereal crops and is further discussed in Chapter 4.2.

Validation of the estimated critical DTPA Zn at the experimental sites: The soil properties were determined for each site and the critical Zn$_{DTPA}$ values were determined from Equation 3 (Chapter 2.2). The critical Zn$_{DTPA}$ values are shown in Table 4.1. For all experimental sites, the measured Zn$_{DTPA}$ values were greater than the critical Zn$_{DTPA}$ (compare the critical Zn$_{DTPA}$ from each site of Table 4.1 to measured Zn$_{DTPA}$ values of Table 4.2, 4.3, and 4.4). That is, the previously applied Zn at these sites had raised the Zn$_{DTPA}$ values to above that required for maximum grain yield of wheat. The critical Zn$_{DTPA}$ values were all greater than the Zn$_{DTPA}$ values of soil that was collected from nearby sites of unfertilised, uncleared vegetation areas that had never been fertilised. That is, the soil test Zn adequately predicted that Zn would have been deficient on the virgin site, and that Zn was still sufficiently supplied in the soil of each experimental site after the prior ZnO application. Unfortunately no experimental sites were located on soils
where Zn had not been previously applied to verify that the model for estimating critical \( Zn_{DTPA} \) adequately predicted grain yield increases to fertiliser Zn.

Although the soil \( Zn_{DTPA} \) values were adequate, several sites had very low Zn concentrations in both the young plant leaves (YEB) and the grain produced. This was particularly so for previously applied Zn and where the plants grown were grown at the highest level of N had been applied. There are several possible reasons for these observed effects on Zn nutrition that a soil test does not take into account. One possible reason is the regular use of many herbicides that may have reduced root growth (“root pruning” effect) which would be expected to inhibit uptake of Zn (Robson and Snowball 1989, 1990). The “pruned” roots have a reduced capacity to explore the soil (Dong et al. 1995) which limits interception of Zn in soil by plant roots thereby reducing uptake by roots from soil Zn. The addition of freshly applied Zn in this study would have increased the number of Zn particles through the regions of soil explored by plant roots greatly enhancing uptake of Zn from soil. The addition of N fertiliser increased soil N supply promoting growth and increased the requirement of the plant for Zn (Loneragan and Webb 1993). The combined effect of high N soil supply and “root pruning” herbicide could quite possibly lower the concentration of Zn to critical levels in both the YEB and the grain without decreases in grain yield. Although the Zn concentrations were at the critical levels, the reasons why maximum yield was reached are not fully understood. However, seasonal growing conditions may have influenced the maximum yield potential the crops could reach and Zn may have been adequate for these yields.

4.1.6 Conclusions

The results of this study show that where the recommended Zn application was originally applied to the soil, and there was regular use of Zn-contaminated superphosphate (to supply P to wheat), applications of high levels of N fertiliser to crops did not induce Zn
deficiency for grain yield of wheat. The soil test Zn adequately predicted that Zn was sufficiently supplied in the soil in all cases. Consequently, further application of Zn fertiliser is not warranted where superphosphate at 150 kg/ha/yr, with Zn as a contaminant (400-600 mg/kg), has been used. However, with high N, and where there is marginal Zn supply in the soil, Zn concentration in the YEB and grain were sometimes close to critical values for these plant parts. The use of compound fertiliser, such as DAP, often with low Zn concentration, could result in Zn deficiency either immediately or after several years of cropping. The use of DAP in cropping and its effects on residual value of Zn is the subject of the next section (Chapter 4.2).
4.2 Residual value of Zn fertiliser for wheat using diammonium phosphate (DAP)

4.2.1 Abstract

In WA, particularly on the acidic sandy soils, Zn deficiency is common in wheat grown with DAP that contains low levels of Zn contamination. The relative effectiveness of Zn fertiliser [Zn oxide (ZnO)] for grain production of wheat was measured in 1996, for Zn applied, either in 1996 (current Zn) or in a previous year (previous Zn) (1983, 1984, 1986, 1990, 1992). In addition, 2 superphosphate treatments (contaminated with 600 mg Zn/kg) were included. Firstly, superphosphate was applied annually to supply the same amount of P as applied by DAP in other plots. The second superphosphate treatment was the same, but in 1983 only, 1.5 kg Zn/ha, as ZnO, was also applied.

Relative to current Zn applied with DAP, the effectiveness of previous Zn for dry weight yield, Zn uptake [Zn concentration multiplied by yield] and grain yield of wheat decreased, the decline in the effectiveness being proportional to the elapse of time since the initial Zn application. Thirteen years after application, the effectiveness was about half that of current Zn for dry weight and grain production where wheat was grown with DAP. Nevertheless, both currently and previously applied Zn fertiliser increased dry weight, Zn content of the dry weight and grain yield of wheat. Zinc applied as a contaminant in superphosphate in the 2 superphosphate treatments produced wheat grain yields on the maximum grain yield plateau (about 2.4 t/ha) achieved for the 5 amounts of ZnO applied in the current year (1996).

Critical concentration of Zn in the youngest emerged leaf blade (YEB) at booting (Gs45) and grain for diagnosing Zn deficiency was 12 mg Zn/kg. Relating the Zn concentrations in the YEB to the grain yield (prognosis), the critical value was 14 mg Zn/kg.

An earlier version of this section was published as “Residual value of zinc fertilizer for production of wheat”. RF Brennan (2001), *Aust. J. Expt. Agric.* 41, 541-547.
4.2.2 Introduction

An initial application of Zn as ZnO has been sufficient to meet the Zn requirements of wheat for many years (residual value) in WA, especially where >150 kg/ha of Zn-contaminated superphosphate was applied annually (Chapter 4.1).

The residual value of Zn applied for wheat sown with DAP on the neutral to acidic soils in WA is not known. In contrast, the residual effectiveness of fertiliser Zn for wheat sown with only superphosphate was studied in Chapter 4.1. Farmers also need to know the length of time that Zn applications as ZnO applied remain fully effective for profitable wheat grain production when crops are sown with DAP fertiliser.

This section examines the results of a long-term field experiment conducted to assess the effectiveness of the original applications of Zn applied to the soil up to 13 years earlier. Zinc was supplied either as ZnO applied with DAP 13, 12, 10, 6 and 4 years previously or as Zn-containing superphosphate [600 mg Zn/kg] applied annually to supply the same P as DAP in the non-superphosphate treatments. In 1983 only, either no ZnO or 1.5 kg Zn/ha as ZnO was applied as the superphosphate treated plots. The experiment compared the effectiveness for grain production of wheat of Zn applied in previous years relative to the effectiveness of Zn applied in the current year.

4.2.3 Materials and Methods

Soil and Site: The field experiment started in 1983 on newly cleared, acutely Zn-deficient soil. The experiment was located 65 km northeast of Esperance [33.47°S, 121.54°E], about 650 km south-east of Perth. The district has a Mediterranean climate, an annual average rainfall of 400 mm, and a growing season (May to October) rainfall of about 300 mm. The current assessment was done in 1996, and monthly rainfall from January to December of that year was (mm): 7, 9, 16, 18, 35, 65, 80, 64, 62, 29, 16, 7, giving a total
of 410 mm and a growing season rainfall of 335 mm.

The experimental site was gravelly sand over clay, and is a yellow duplex soil, classified as Dy5.82 (Northcote 1979) or as Fluventic Xerochrept (Soil Survey Staff 1975). Some properties of the top 10 cm of the <2mm fraction of soil, as measured on samples collected before the experiment started, were: pH 5.3 (1:5 soil:solution 0.01 M CaCl₂); clay, 7 %; silt, 9 % (Day 1965); organic carbon, 1.1 % (Walkley and Black 1934); cation exchange capacity, 6.9 cmol/kg (Gillman and Sumpter 1986); Zn DTPA <0.2 mg Zn/kg (Lindsay and Norvell 1978); and bicarbonate-extractable P (Colwell 1963), 6 mg P/kg.

**Experimental procedures:**

*(1) History of the experiment:* The experimental design was a randomised complete block with 3 replications. There was 0.8 m of untreated soil between each 4.2 by 50-m plot. Three sources of Zn were applied in 1983:

1. Zn applied once only at 0, 0.5, 1.0, 1.5, and 3.0 kg Zn/ha as ZnO.

2. Zn present in superphosphate (600 mg Zn/kg) applied to supply equivalent amounts of P applied as DAP to the non-superphosphate treatments. In all subsequent years, the amount of superphosphate applied matched the P applied annually as DAP to the non-superphosphate treatments. In the superphosphate treatments the Zn contamination present in the superphosphate was therefore also applied annually.

3. Superphosphate applied as just described in (2), but in 1983 1.5 kg ZnO/ha was also applied with the superphosphate.

The recommended amount of Zn applied to wheat grown on the soil type is 1.5 kg ZnO/ha (Gartrell and Glencross 1968). Zinc treatments were placed (drilled) 5 cm deep with the seed of wheat while sowing. Wheat was sown in 1983, 1984, 1986, 1990, 1992, and in
1996, and in these years, 0.5, 1.0, 1.5 and 3.0 kg Zn/ha, as ZnO, was applied to plots not treated with Zn fertiliser in a previous year. Clover (cv. Daliak) was sown in 1985, and naturally regenerated clover grew in all the other years when wheat was not grown and no fresh Zn was applied to plots. Basal fertilisers were applied to all plots in all years from 1983 to 1996, including the many nil-Zn plots yet to be treated with ZnO in a subsequent year, to ensure no nutrient element, except Zn, limited plant yield. Fertiliser phosphorus was applied each year at 25 kg P/ha as imported DAP (except in treatment 2) which contained < 50 mg Zn/kg. The ZnO treatments were applied with DAP fertiliser at equivalent amounts of P as the superphosphate treatments. Similarly, the equivalent amount of N supplied in the DAP fertiliser was applied to the superphosphate plots as ammonium nitrate. That is, there were no differences in the amounts of P, S and N applied to any of the plots. Gypsum (17 % S) was applied with DAP.

(2) The experiment in 1996: In 1996, wheat at 70 kg seed/ha was sown in the previously established separate 4.2 by 50 m plots for each previous or current Zn treatment. Twenty four rows of seeds, 175 mm apart, were sown down each plot in mid-May.

To ensure that Zn was the only nutrient limiting wheat yield, basal fertilisers were applied to all plots (kg fertiliser/ha): (1) CoSO₄.7H₂O (0.25 kg/ha; 22 % Co), Na₂BO₄ (3.0 kg/ha; 8 % B), applied to the soil surface immediately before sowing; (2) CuSO₄.5H₂O (6.0 kg/ha; 25 % Cu), MnSO₄.5H₂O (12.0 kg/ha; 25 % Mn) and Na₂MoO₄.2H₂O (0.25 kg/ha; 39 % Mo) placed (drilled) with the seed and (3) KCl (70 kg/ha, 50 % K), urea (125 kg/ha, 46 % N) and gypsum (150 kg/ha; 17 % S) applied to the soil surface 4 weeks after emergence (mid-June). The total amount of N applied to all plots was 75 kg N/ha, P was applied at 25 kg P/ha and a total of 45 kg S/ha was also applied.

All weeds were successfully controlled using pre and post-emergent herbicides. Insects were controlled with pesticides as required.
Measurements (1996): At the 2 to 3-leaf growth stage (Gs12-13, Zadoks et al. 1974), 21 days after sowing, wheat seedlings were counted along 1-m lengths of 2 adjacent rows at 10 random positions within each plot. The dry matter production of wheat plants (shoots) was measured in September (Gs59) by cutting 35 plants at soil level, from random locations within the middle 20 rows [excluding 2 rows on each edge] of each plot. The Zn concentration in youngest emerged leaves (YEB) of wheat plants were measured in September by sampling 35 plants, from random locations within the middle 20 rows of each plot from Zn applied in 1983, 1990 and 1996.

In order to avoid contamination with Zn from the harvesting machine, 50 to 55 plants were collected at random locations within the middle 20 rows of each plot from Zn applied in 1983, 1990 and 1996. Replicate samples of dry matter and grain were ground and digested in a nitric-perchloric acid mixture and the Zn concentration in the digest was analysed by atomic absorption spectrophotometry (Allen 1961).

Grain yield was measured by machine harvesting grain from the middle 20 rows of each plot in early-December and weighed.

Analysis of Data: The relationship between yield of dried shoots or grain yield and the amount of Zn applied was fitted with a Mitscherlich equation as described by Barrow and Mendoza (1990):

\[ y = a - b \exp(-c x) \]  

(1)

where \( y \) is the yield of dried shoots or grain yield (t/ha), \( x \) is the amount of Zn applied (kg Zn/ha) and \( a, b, \) and \( c \) are coefficients. Coefficient \( a \) provides an estimate of the asymptote or maximum yield plateau. Coefficient \( b \) (kg/ha) estimates the difference between the asymptote and the intercept on the yield axis at \( x = 0 \). Therefore, \( b \) indicates the maximum increase in yields (yield response) due to the application of Zn.
fertiliser. Coefficient $c$ (ha/kg Zn) describes the shape of the relationship and governs the rate at which $y$ (the yield response) increases as $x$ (the amount of Zn applied) increases (Ratkowsky 1990). Mean data were fitted to the equation by non-linear regression using a computer program written in compiler BASIC (Barrow and Mendoza 1990). The simplex method (Nelder and Mead 1965) was used to locate the least squares estimate of the non-linear coefficients.

For production of shoots and grain, the effectiveness of Zn applied in each of the previous years was calculated relative to the effectiveness of Zn applied in the current year (1996), to provide residual values (RV$_{Zn}$). As previous and current Zn treatments attained the same maximum yield, this was done by dividing $c$ values of Zn applied in the current year (1996) or each of the previous years (1983, '84, '86, '90 and '92) by $c$ for Zn applied in the current year (1996) (Barrow and Campbell 1972); therefore, by definition, the RV$_{Zn}$ for Zn applied in the current year is always 1.00.

For Zn uptake (yield of dried shoots multiplied by Zn concentration in the shoots), different maximum Zn uptake was reached for Zn applied in 1996, 1990 and 1983. It is therefore not valid to use the $c$ coefficient of the Mitscherlich equation to compare responses to applied Zn fertiliser (Barrow and Campbell 1972; Barrow 1985). Instead, the initial slope of the relationship between Zn uptake and the amount of Zn applied was used to compare the uptake response of the wheat to Zn application. For the Mitscherlich equation, as $x$ approaches zero, $dy/dx$ approaches $bc$ so that $bc$ was used as an estimate of the initial slope (Barrow and Campbell 1972; Barrow 1975).

The relationship between dry weight or grain yield (dependent variable, $y$ axis), and the concentration of Zn in YEB (independent variable, $x$ axis), was used to define critical Zn concentrations in YEB. The critical value is the Zn concentration in YEB that corresponded with 90 per cent of the maximum, Zn non-limiting yield (Ulrich and Hills
1967). The value of the $a$ coefficient of the Mitscherlich equation fitted to the relationship between yield and the amount of Zn applied was used as the maximum yield. In this study, the Mitscherlich and hand drawn curves were used to determine critical Zn concentrations in YEB that were related to 90 per cent of the maximum yield of dry weight or grain.

4.2.4 Results

Analysis of variance indicated highly significant ($P<0.001$) effects on yield of shoots, grain and Zn uptake due to amounts of Zn addition and significant ($P<0.05$) effects due to year of application and the interaction of the amount of Zn applied by year of application.

**Plant density:** Additions of fertiliser Zn in the current or previous years did not have an effect on plant emergence. The plant density of wheat seedlings 168 plant/m$^2$ (standard error, s.e. = 12.8; $n = 96$), which is an adequate density for maximum wheat grain production in WA (Anderson et al. 2000).

**Zn deficiency symptoms:** Symptoms of Zn deficiency, as chlorotic stripes each side of the mid–rib on younger leaves, was observed on plants grown without added Zn (nil Zn) and those plots with low amounts of Zn applied 13 years previously. Zinc-deficient plants were severely stunted, chlorotic with the middle sections of leaves collapsing while plants took on a “water-soaked” appearance. By the start of flowering (mid-September), many of the wheat plants on the nil Zn treatment plots had died due to Zn deficiency.

**Shoot yield:** Applications of fertiliser Zn in the current or previous years increased the dry weight of shoot (DWS) of wheat (Figure 4.1a).

(a) **Zinc as superphosphate:** In 1996, both superphosphate treatments produced DWS yields on the maximum yield plateau achieved for ZnO applied in the current year (1996). For example, Zn containing superphosphate produced about 5.6 t/ha and
1.5 kg ZnO/ha applied in 1996 produced 5.7 t/ha. The Zn applied each year in superphosphate, therefore provided adequate Zn for maximum DWS yield [about 5.5 t/ha] of the wheat. Similarly, as in Chapter 4.1, an initial ZnO application of 1.5 kg/ha supplemented by annual additions in superphosphate was adequate for maximum yield.

(b) Zinc applied as ZnO: For simplicity and clarity, data are only shown for the DWS yield increase results from ZnO applied in 1983, 1990 and 1996 (Figure 4.1a). Zinc applied at 3.0 kg/ha in 1983 with DAP produced DWS yields on the maximum yield plateau achieved for 1.5 kg/ha of Zn applied in the current year (1996). All other years fitted into the pattern established by 1983, 1990 and 1996 (Figure 4.1a).

(c) Zinc oxide effectiveness in current and previous years (RVZn values): The Mitscherlich equation adequately described all the DWS increases to freshly and previously applied Zn (Figure 4.1a, Table 4.5). Relative to current Zn, there was a steady decline in the effectiveness of Zn as time since the Zn was applied increased. That is, the effectiveness of the Zn decreased the longer the Zn was in contact with soil (Figure 4.2, RVZn values in Table 4.5). For example, relative to the Zn applied in 1996 (the current year), the Zn applied as ZnO 13 years previously was about 50% as effective for shoot yields of wheat (see the RVZn values in Table 4.5). This means that about double the amount of Zn applied 13 years ago was required to produce the same yield as current Zn applied in 1996.

(d) Zinc content in shoots: Current and previously applied Zn increased the Zn content of wheat shoots (Figure 4.1b). Data were only collected for 3 years of Zn application (1983, 1990 and 1996) (Figure 4.1b). A Mitscherlich equation adequately described the Zn uptake of shoots in response to freshly and previously applied Zn for each of the 3 years.
Table 4.5. Values of the coefficients of the Mitscherlich equations fitted to the relationship between shoot dry weight (t/ha), Zn content (g/ha) of the dry matter or grain yield (t/ha) and the amount of Zn fertiliser applied with DAP. Residual value ($RV_{Zn}$) of Zn also calculated. Coefficient of determination ($R^2$) > 0.9 for all cases.

<table>
<thead>
<tr>
<th>Yrs$^a$</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>bc</th>
<th>$RV_{Zn}$ $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry weight of shoots</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>5.42</td>
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</tr>
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<td>12</td>
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</tr>
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<td>1.17</td>
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<td></td>
</tr>
<tr>
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<td>5.5</td>
<td>4.17</td>
<td>1.48</td>
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</tr>
<tr>
<td>4</td>
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<td>4.27</td>
<td>1.69</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5.98</td>
<td>4.68</td>
<td>2.16</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zn content</td>
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</tr>
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<td>67.6</td>
<td>0.43</td>
</tr>
<tr>
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<tr>
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<td>156.7</td>
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</tr>
<tr>
<td></td>
<td>Grain yield</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>13</td>
<td>2.26</td>
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<td>1.05</td>
<td>0.49</td>
<td></td>
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<td>1.77</td>
<td>1.06</td>
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<tr>
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<td>1.78</td>
<td>1.1</td>
<td>0.52</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>2.33</td>
<td>1.83</td>
<td>1.42</td>
<td>0.67</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.36</td>
<td>1.8</td>
<td>1.6</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>2.4</td>
<td>1.9</td>
<td>2.12</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Years since Zn applied, 1996=0; 1992 =4 years; 1990 = 6 years; 1986=10 years; 1984=12 years; 1983=13 years.

$^b$The residual value (RV) was calculated using the dry weight of shoots or grain yield by dividing the c coefficient of each previous and current year (YR 0) by the c coefficient of the current year. Therefore, by definition $RV_{Zn}$ for Yr 0 is 1.00. Similarly, for the RV calculated using the uptake (content of the dry matter) was determined by dividing the bc values for each year by the bc value for the yr 0. Therefore by definition the $RV_{Zn}$ for yr 0 is 1.00.

As measured using Zn uptake, relative to current Zn, there was a steady decline in the effectiveness of Zn with the length of time since the Zn was applied. That is, the effectiveness of Zn decreased the longer it was in contact with soil ($RV_{Zn}$ values in Table 4.5). For example, relative to the Zn applied in the current year, Zn applied 13 years previously was about 43% as effective (see $RV_{Zn}$ values in Table 4.5).
Figure 4.1 The relationship between (a) shoot dry matter, (b) Zn uptake of shoots or (c) grain yield for wheat grown in 1996 with DAP and the amount of Zn applied ZnO was applied in 1983 (■), 1990 (▲), or 1996 (♦). Fitted regression equation coefficients shown in Table 4.5.
Figure 4.2 The decline in effectiveness of Zn applied as ZnO with DAP in each year for shoots (♦) or grain yield (■) relative to the effectiveness of Zn applied in the current year (residual value or RV_{Zn}) with length of time since the Zn fertilizer had been applied to the soil. The fitted curve: $RV=0.349 + 0.654\exp(-0.116x)$; $R^2 = 0.92$.

Grain Yield

(a) **Zinc as superphosphate**: Zinc applied as a Zn contaminant in superphosphate annually, coupled with either zero or 1.5 kg ZnO applied in 1983 only, produced wheat grain yields on the maximum grain yield plateau [about 2.4 t/ha] achieved for the ZnO applied in the current year (1996).

(b) **Zinc applied as ZnO**: For clarity, data are only shown for 1983, 1990 and 1996 (Figure 4.1c). Current and previously applied Zn with DAP increased grain yield of wheat (Figure 4.1c). Zinc applied at 3.0 kg/ha in 1983 with DAP produced grain yields on the maximum yield plateau achieved for 1.5 kg/ha of Zn applied in the current year (1996).

(c) **Zinc oxide effectiveness in current and previous years (RV_{Zn} values)**: A Mitscherlich equation adequately described all the grain yield increases to freshly and
previously applied Zn (Table 4.5). Relative to current Zn, there was a steady decline in the effectiveness of Zn for grain production as the length of time since the Zn was applied increased (Figure 4.2, $RV_{Zn}$ values in Table 4.5). For example, relative to the Zn applied in the current year, the Zn applied 13 years previously was about 50% as effective (see the $RV_{Zn}$ values in Table 4.5).

_Zinc concentrations in the youngest fully emerged leaf and grain:_ Zinc concentrations in the YEB at Gs45 from the nil-Zn treatment were about 7 mg Zn/kg (see bottom left data point of Figure 4.3). Zn concentration of the YEB increased with each amount of Zn applied in the current and in previous years. Generally, as the length of time increased that the Zn application had been in contact with the soil, the increase in the concentration of Zn in the YEB reduced.

There was a good relationship between the concentration of Zn in the YEB with dry weight of shoots (Figure 4.3a) and with grain (Figure 4.3b) yield. From the relationship between shoot dry weight and the concentration of Zn in the YEB, the critical diagnostic Zn concentration was about 12 mg/kg. Similarly, for the relationship between grain yield (dependent variable or y axis) and the concentration of Zn in the YEB (independent or x axis), the prognostic critical Zn concentration was about 14 mg Zn/kg.

The critical Zn concentration determined from the hand-fitted curves were the same as those calculated from the Mitscherlich equation (Figure 4.3a, b). Zinc concentrations in the grain from the nil-Zn treatment were about 8 mg Zn/kg but increased with Zn additions (see bottom left data point of Figure 4.3c). Zn concentration of the grain increased with each level of applied Zn, but decreased the longer Zn was in contact with soil.
Figure 4.3 The relationship between (a) dried shoots weight and Zn concentration in the YEB, (b) grain yield and Zn concentration in the YEB, and (c) grain yield and Zn concentration in the grain for wheat grown in 1996 when Zn was applied in 1983 (■), 1990 (▲), and 1996 (●). Solid curves are for Mitscherlich equation; dashed lines are for hand-fitted curves. The fitted Mitscherlich relationship: (a) $y = 5.82 - 45.97 \exp(-0.33x)$; $R^2 = 0.98$; (b) $y = 2.33 - 18.90 \exp(-0.336x)$, $R^2 = 0.98$; (c) $y = 2.32 - 109 \exp(-0.513x)$, $R^2 = 0.99$. 
There was a good relationship between grain concentration of Zn and grain yield (Figure 4.3c). From the relationship between grain yield (dependent variable or y axis) and the concentration of Zn in the grain (independent or x axis), the critical diagnostic Zn concentration for grain was about 12 mg/kg. The critical diagnostic concentration of Zn in grain determined from the hand-drawn curve was the same as calculated from the Mitscherlich (Figure 4.3c). This critical diagnostic concentration of Zn in grain is consistent with other published data (Brennan et al. 1993).

4.2.5 Discussion

Relative to fresh ZnO applied in 1996, the level of Zn contamination in the superphosphate applied annually for 13 years supplied adequate Zn for wheat. Likewise, the superphosphate applied annually since 1983 when 1.5 kg Zn/ha as ZnO was applied, had a good residual value 13 years after application. This result is consistent with results of previous research for cereal crops (Takkar and Walker 1993; Chapter 4.1). Previous research with cereal crops has shown that the use of superphosphate containing Zn when applied at > 150 kg/ha has supplied amounts of Zn to meet the requirements of the current crop, maintaining adequate soil Zn levels despite any decline in the effectiveness of the original Zn application (Brennan 1998; Chapter 4.1). The residual effectiveness of Zn fertiliser is due to (i) undissolved Zn still present in the fertiliser, (ii) Zn that has dissolved from the fertiliser which is either retained by the soil, recycled by the plants as organic matter or recycled in animal excreta. Barrow (1980) has described these soil and plant processes for phosphorus. The amount of Zn removed in product is typically low relative to the amount of Zn applied. In the present study, where 3 kg Zn/ha had been applied to the soil in 1983, about 7 % of the total Zn was removed in the grain (the product of grain yield and the Zn concentration in the grain) from all the subsequent wheat crops (the summation of the Zn content) up to 1996. The pasture was not grazed or defoliated through the time of the present study so negligible Zn was removed in the pasture years.
Losses of Zn from the soil system through leaching (Chapter 5) and erosion (see Chapter 7) are negligible. Therefore, most of the residual Zn would still present in the soil. In the present study, Zn applied in 1983 had been in contact with the soil for the longest period and therefore had most time for the slow reactions between the soil and Zn to occur. These processes have been shown to decrease the effectiveness of Zn added to soil (Barrow 1987; see Chapter 2). In addition, Zn applied in previous years was increasingly incorporated throughout the soil when sowing crops with tined machines, which may increase retention of Zn by the soil but also improves interception of Zn in the soil by roots (Gartrell 1981; Mortvedt and Gilkes 1993). The Zn fertiliser is then in contact with a greater soil volume allowing chemical reactions (sorption) to take place which will further diminish the availability of the applied Zn for plant uptake. These reactions have been demonstrated to take place and these results are consistent with findings of Chapter 2. The net effect of on-going Zn sorption and increased root interception of Zn in the soil is a decrease in effectiveness of Zn relative to freshly applied Zn for plant production and Zn uptake by plants.

The residual effects of Zn application are not as long lasting for soil types and plant species other than those studied here (Takkar and Walker 1993). For example, Weir and Holland (1980) found that an application at 18 kg Zn/ha applied to a black earth (higher pH and clay content than the soil used in this study) declined in effectiveness of Zn for maize production after ten years. Takkar et al. (1975) suggested that an application of 22 kg Zn/ha to a loamy sand would be sufficient for at least seven crops in a wheat-groundnut rotation, a conclusion based on observing the decline in soil-extractable Zn over three crops. The residual effectiveness for maximum grain production has often been less than 10 years in alkaline and sodic soils (Martens and Westermann 1991; Takkar and Walker 1993).

In the present study, both the Mitscherlich method and hand-fitted curves were used to
determine critical diagnostic Zn in shoots and grain and both resulted in similar critical concentrations of Zn. In previous studies, when the Mitscherlich equation has been fitted to the relation between yield and the concentration of an element in plant tissue to determine critical concentrations, significantly higher and more variable critical concentrations of nutrient elements have been calculated (Ware et al. 1982; Bell et al. 1990; Wilhelm et al. 1993). By contrast, hand-fitted curves have given more consistent critical concentrations and so have often been used to determine critical nutrient concentrations (Reuter et al. 1983; Wilhelm et al. 1993; Khan et al. 1998). Zinc concentration in YEB is used as a method of determining Zn deficiency (diagnosis) of wheat. Concentrations of 12 mg Zn/kg in the YEB suggest that the wheat crop is Zn deficient and future applications of Zn fertiliser are required (Brennan et al. 1993). The critical level of Zn in YEB to diagnose Zn deficiency in wheat is consistent with other published data (Brennan 1992; Brennan et al. 1993) but lower than the 18 mg Zn/kg in the YEB of wheat suggested by Wilhelm et al. (1993). The critical concentration of 14 mg Zn/kg in the YEB for predicting effects on grain yield agrees with other data (Riley et al. 1992; Brennan et al. 1993). Zinc concentration in grain can be used as a post-mortem method of determining Zn deficiency of wheat; concentrations of 12 mg Zn/kg in wheat grain suggest future applications of Zn fertiliser are required (Brennan et al. 1993).

Zinc concentration of 12 mg Zn/kg in young leaves of wheat is used to diagnose Zn deficiency (Brennan et al. 1993) and is now being used to provide fertiliser recommendations for farmers in WA. If Zn deficiency is diagnosed before stem elongation, a foliar application of Zn can be made albeit with some yield loss compared to supplying Zn at seeding (Chapter 3.2). Zine sulfate is widely used as a foliar spray in situations where Zn deficiency has been diagnosed in the growing crop. Zinc foliar sprays need to be applied at an early growth stage of cereals (Duncan 1967; Sharma and Katyal 1986; Chapter 3.2). Soil application of Zn with seed resulted in the highest grain
yield, whereas foliar application of Zn sprayed early in the vegetative stage resulted in grain yield losses due to early Zn deficiency (Chapter 3.2).

Zinc concentration in the grain is of less value in determining Zn deficiency than Zn concentration in YEB (Brennan et al. 1993). Using grain analysis to indicate Zn deficiency could be misleading because Zn deficiency early in the growing season would have reduced tiller production and the redistribution of Zn to the reduced grain sink could result in reasonable levels of Zn in the grain. However, Zn levels of <12 mg Zn/kg in wheat grain may suggest future Zn fertiliser is required. In this study, no further grain yield increase with Zn fertiliser was found where grain Zn concentration was 12 mg/kg. Data from the field suggest critical Zn concentration in the grain was 9 to 10 mg/kg (Riley et al. 1992).

4.2.6 Conclusions

The level of Zn contamination in the superphosphate has greatly influenced the Zn status of soils of WA (Brennan and Gartrell 1991). Although the application of Zn to the soils of this region is low (0.6 to 2.6 kg Zn/ha), the annual use of superphosphate at >150 kg/ha has supplied sufficient amounts of Zn (~90 g Zn/ha per year) to meet the requirements of the current crop, maintaining adequate soil Zn levels. The application of about 90 g Zn/ha per year as part of annual superphosphate applications is a “maintenance” application of Zn required for producing about 3 t/ha of wheat grain. The maintenance Zn application has masked any decline in the effectiveness of the original Zn application as ZnO (Brennan and Gartrell 1991; Chapter 2).

The results of this study show that where the recommended Zn application was originally applied to the soil, applications of high levels of N fertiliser to cereal crops did not increase the incidence of Zn deficiency of the wheat crops. Consequently, further application of Zn fertiliser is not warranted where superphosphate, with Zn as a
contaminant, has been used. The use of compound fertiliser, such as DAP, often with low Zn concentration, could result in Zn deficiency either immediately or after several years of cropping (Brennan and Gartrell 1991). The length of time before Zn deficiency is observed after using DAP would depend on the initial level of an application of Zn, how long since it was applied and the history of superphosphate applications to legume crops or pastures between the cereal phases.
Chapter 5

Residual Effectiveness of Zinc Fertiliser in Soils

5.1 The vertical movement of Zn on sandy soils in Western Australia

5.1.1 Abstract

Previous studies report widely different conclusions about leaching of Zn additions to soils. In order to determine the significance of leaching for residual effectiveness (RE) of Zn fertiliser in WA, the present study assessed the movement of Zn vertically down the profile of sandy soils by both soil chemical extraction and radioisotope techniques. The movement of Zn vertically down the soil profile of grey sand of low clay content (<3%), acidic (pH<sub>Ca</sub> 4.7), and low cation exchange capacity (4.1 cmol (+)/kg) was measured in 2 field experiments located in the high rainfall (1100 mm annual average) region of WA. Zinc fertiliser was applied to the surface. At levels of Zn typically used in agriculture and forestry (0.7 to 1.5 kg Zn/ha) in WA there was little movement of Zn below 2.5 cm even after 1438 mm of rain. Where Zn was applied at 22.5 kg/ha, about 95% of the applied Zn could be accounted for in the top 5 cm of soil. At the highest Zn application (68 kg Zn/ha), 37% of the applied Zn was recovered in the 5–15 cm soil sampling depth. There was little difference in the movement downwards between the soluble sulfate source, and the insoluble oxide source of Zn. The negligible movement of Zn below the depth of placement in a very sandy soil in high rainfall areas suggests that loss of Zn from the rooting zone of most agricultural plants grown in WA is negligible.

An earlier version of this chapter was published as “The vertical movement of zinc on sandy soils in southern Western Australia”. RF Brennan, McGrath JF (1988), *Aust. J. Soil Res.*, 26, 211 – 216.
5.1.2 Introduction

Little movement of Zn down the soil profile has been reported in most studies, but some studies have reported considerable movement (leaching) of Zn down soils of varying texture (Barrows et al. 1960; Keefer and Estepp 1971; Abebe 1972; Singh and Shukla 1976; Novillo et al. 2002; Obrador et al. 2003). Jurinak and Thorne (1955) found that Zn applied as a chloride (soluble source) to the surface of silty clay moved less than 3 cm under strongly leaching conditions. Similarly, Novillo et al. (2002) showed little movement of Zn, applied as organic Zn complexes, to soils ranging from acidic to calcareous. Obrador et al. (2003) showed little movement of Zn in a calcareous soil. Brown et al. (1962) showed that Zn applied as either the oxide (insoluble source) or sulfate (soluble source) did not move appreciably either in columns of a sandy loam or a silt loam soil that were leached.

In radioisotope studies, Singh (1974) showed that most of the Zn applied (about 95 %) was retained in the upper 3 cm of a loamy sand although there was some movement (<4 %) of Zn to 12 to 18 cm. In contrast, 2 radioisotope studies showed considerable movement of Zn down soils. Barrows et al. (1960) measured movement of Zn to 45 cm for a sandy soil. Abebe (1972) showed that a surface application of $^{65}$Zn to a sandy loam was leached to a depth of 30 cm by the equivalent of 114 mm of surface water; however, the highest concentration of Zn was retained in the upper 4 cm of the soil.

The contrasting reports on the movement of Zn in different soil types and with varying methods of leaching in soil columns (e.g. Abebe 1972; Singh and Shukla 1976; Novillo et al. 2002; Obrador et al. 2003) has led to the present investigation. Since Zn deficient soils occur over a wide range of rainfall zones in WA (Gartrell and Glencross 1968), there is a need to know if in higher rainfall areas Zn is transported (leached) beyond the root zone of plants as this will diminish the residual value of Zn fertiliser. The extent of
leaching of Zn on the sandy soils of WA has not been determined.

As a high rainfall area (> 1000 mm annual average rainfall) was sown to *Pinus radiata* D. Don seedlings, the opportunity was taken to measure Zn leaching. *Pinus radiata* D. Don requires Zn fertiliser for adequate growth (Smith and Bayliss 1942; Raupach 1975). In this study the leaching of Zn applied in the field to the surface of a sandy soil in the high rainfall zone of WA was determined. These conditions of high rainfall and sandy soil types represent the highest risk of Zn leaching in WA. The soil had not been previously fertilised with Zn or other essential plant nutrient elements. Both radioisotope addition and chemical extraction of Zn were used to provide information on Zn movement in this soil type.

### 5.1.3 Materials and Methods

A total of 3 field experiments located at two sites, east of Busselton (mean annual rainfall 1100 mm), were established on areas of soil with no previous fertiliser application from which the native jarrah forest had been cleared in the previous year. One-year-old *Pinus radiata* D. Don seedlings were planted in June at both sites. The seedlings were planted at 2.5-m intervals on rows 3.5 m apart. Fertiliser Zn was applied to plots of 1 m² area that were centred on the pine seedlings.

Surface soil (0 -10 cm) of the grey sand (about 95 % sand) (Uc2.22, Northcote 1979) from the two virgin sites was characterised (Table 5.1). Soil pH<sub>Ca</sub>, clay content (%), sesquioxides (Fe₂O₃ & Al₂O₃) using a modification of Coffin's procedure (Hesse 1971), and organic carbon (OC) were determined as outlined in Chapter 2.1.3. Total soil-Zn concentration was determined by atomic absorption spectrophotometry after digestion in perchloric and hydrofluoric acids. Soil extractable Zn were measured by DTPA (Zn<sub>DTPA</sub>) (Lindsay and Norvell 1978) and ammonium oxalate (NH₄OX) extractable Zn based on the Gupta and McKay (1966) procedure for copper.
**Experiment 1:** Experiment 1 consisted of several levels of Zn, as the soluble sulfate source, applied at 2 sites. At site 1, Zn sulfate (22.7 % Zn) was applied to plots as a solution (500 mL/m²) in October 1979. Three levels of Zn sulfate were applied; 0, 1.1 and 2.25 kg Zn/ha to the soil surface of plots of 1 m² area.

At site 2, a similar experiment with higher Zn applications was conducted in July 1980. Zinc sulfate was applied to the surface of the soil at levels of 0, 22.5 and 68 kg Zn/ha. The Zn sulfate for each level was mixed with 400 g of dried soil that had received no previous Zn and was spread evenly over the surface of 1 m² area plots.

**Table 5.1.** Properties of the acid grey sand at site 1 and site 2 located east of Busselton (mean annual average rainfall 1100 mm).

<table>
<thead>
<tr>
<th></th>
<th>pH&lt;sub&gt;Ca&lt;/sub&gt;</th>
<th>Clay (%)</th>
<th>OC&lt;sup&gt;a&lt;/sup&gt; (%)</th>
<th>Fe&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt; (%)</th>
<th>Al&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt; (%)</th>
<th>CEC&lt;sup&gt;b&lt;/sup&gt; cmol(+)/kg</th>
<th>NH&lt;sub&gt;4&lt;/sub&gt;OX Extractable Zn (mg/kg)</th>
<th>DTPA Extractable Zn (mg/kg)</th>
<th>Total Zn (mg/kg)</th>
<th>Bulk density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.7</td>
<td>3.5</td>
<td>1.47</td>
<td>0.02</td>
<td>0.06</td>
<td>4.1</td>
<td>0.30</td>
<td>0.23</td>
<td>2.1</td>
<td>1.46</td>
</tr>
<tr>
<td>2</td>
<td>4.8</td>
<td>4.0</td>
<td>1.38</td>
<td>0.03</td>
<td>0.07</td>
<td>4.5</td>
<td>0.32</td>
<td>0.21</td>
<td>2.1</td>
<td>1.45</td>
</tr>
</tbody>
</table>

<sup>a</sup>OC is the organic carbon content (%) measured by Walkley and Black (1934) method.

<sup>b</sup>CEC is the cation exchange capacity of the soil measured according to Tucker (1974).

A basal dressing of copper sulfate (25 % Cu, 10 kg/ha), single superphosphate (9.1 % P; 800 kg/ha, supplied 0.32 kg Zn/ha) and ammonium nitrate (120 kg/ha) was also applied to the soil surface at both sites. Each treatment was replicated four times in a completely randomised design. Within each replicate there were four subplots. Three soil cores (5 cm diameter) were taken at the start of the experiment from each 1 m² subplot and divided into 0-2.5 cm, 2.5-5 cm, and 5-15 cm segments. The soil sampling procedures were repeated at 4 months for site 1 and 12 months for experiment 1 at site 2. The three cores at each sampling site within the replicate of a treatment were bulked but each replicate was individually analysed. Soil samples were oven-dried and analysed for NH<sub>4</sub>OX-extractable Zn, Zn<sub>DTPA</sub> and total Zn.

**Experiment 2:** Radioactive Zn chloride was converted into labelled radioactive Zn (⁶⁵Zn;
244 day half-life) oxide and sulfate. The $^{65}$Zn either as oxide or sulfate was applied to each 1 m$^2$ plot at 0.75 kg Zn/ha. Experiment 2 consisted of 2 sources of Zn and was located on unfertilised soil adjacent to site 1 of experiment 1. The $^{65}$Zn was evenly applied across the soil surface of each plot after being thoroughly mixed in 400 g of dried unfertilised soil. Each treatment was replicated three times. Superphosphate (800 kg/ha) and copper sulfate (5.6 kg/ha) were evenly applied to the soil surface (topdressed) of each plot. Plots were kept free of weeds by herbicide use.

Four core samples of 5 cm diameter each 20 cm deep were taken from each replicate of each treatment. The core samples were taken 4, 18, 32, 59, 89 and 374 days after the initial $^{65}$Zn application. The individual cores of 20 cm were separated into 1.0-cm segments. To minimise possible contamination of lower segments from the upper segments as the soil sampling tool moved from zones of higher $^{65}$Zn concentration to zones of low $^{65}$Zn concentration, each 1 cm segment was re-sampled by inserting a smaller diameter tube (2 cm) through the sample from the lower to upper surface. Each 1 cm segment was measured for residual activity of $^{65}$Zn by using a scintillation counter for 100 s. Counts were corrected for isotopic decay.

5.1.4 Results

*Experiment 1:* There was no movement of Zn into the 2.5-5 cm soil depth (Table 5.2). Although 1225 mm of rain had fallen on the site over the sampling period, there was no detectable increase of soil Zn concentration below 2.5 cm depth when Zn sulfate had been applied to the soil surface at levels of up to 10 kg/ha (Table 5.2). The results suggest that little or no Zn moved more than 2.5 cm down the soil profile. Application of Zn to the soil surface increased the NH$_4$OX extractable Zn and Zn$_{DTPA}$ and total Zn for the 0-2.5 cm soil depth.
Table 5.2. Effect of surface applied Zn as the soluble Zn sulfate on extractable zinc and total Zn levels within a sand profile after 1225 mm of rain in 12 months. *Experiment 1, site 1.* Values are means ± s.e.

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>NH₄OX Soil test Zn extractant:</th>
<th>DTPA Soil test Zn extractant:</th>
<th>Total Zn Soil test Zn extractant:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Extractable Zn. (mg /kg)</td>
<td>Extractable Zn. (mg /kg)</td>
<td>(mg /kg)</td>
</tr>
<tr>
<td>0 - 2.5</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>2.5 - 5</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>5 – 15</td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>ND a</td>
</tr>
<tr>
<td>1.1 kg Zn/ha b</td>
<td>0 - 2.5</td>
<td>3.4 ± 0.6</td>
<td>5.4 ± 0.5</td>
</tr>
<tr>
<td></td>
<td>2.5 - 5</td>
<td>0.3 ± 0.1</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5 – 15</td>
<td>0.3 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>2.25 kg Zn/ha c</td>
<td>0 - 2.5</td>
<td>7.1 ± 0.6</td>
<td>9.2 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>2.5 - 5</td>
<td>0.3 ± 0.1</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>5 – 15</td>
<td>0.3 ± 0.1</td>
<td>2.1 ± 0.2</td>
</tr>
</tbody>
</table>

a ND, not determined.
b Zn at 1.1 kg/ha was applied as the sulfate source of Zn.
c Zn at 2.25 kg/ha was applied as the sulfate source of Zn.

Table 5.3. Effect of surface applied Zn sulfate on the ammonium oxalate, DTPA extractable and total Zn levels (mg/kg) within a sand profile after 575 mm of rain in 4 months. *Experiment 1, site 2.* Values are means ± s.e.

<table>
<thead>
<tr>
<th>Soil profile depth (cm)</th>
<th>Soil test Zn extractant:</th>
<th>NH₄OX Soil test Zn extractant: (mg/ kg)</th>
<th>DTPA Soil test Zn extractant: (mg/ kg)</th>
<th>Total Zn Soil test Zn extractant: (mg/ kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 kg Zn/ha</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0 – 2.5</td>
<td></td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>2.5 – 5.0</td>
<td></td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>5 – 15</td>
<td></td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>22.5 kg Zn/ha a</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 2.5</td>
<td></td>
<td>53.0 ± 7.8</td>
<td>32.2 ± 2.6</td>
<td>57.0 ± 8.7</td>
</tr>
<tr>
<td>2.5 – 5.0</td>
<td></td>
<td>7.3 ± 1.2</td>
<td>4.6 ± 0.4</td>
<td>9.3 ± 0.9</td>
</tr>
<tr>
<td>5 – 15</td>
<td></td>
<td>0.3 ± 0.1</td>
<td>0.2 ± 0.1</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>68 kg Zn/ha b</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 – 2.5</td>
<td></td>
<td>72.3 ± 4.9</td>
<td>44.7 ± 3.2</td>
<td>74.0 ± 8.2</td>
</tr>
<tr>
<td>2.5 – 5.0</td>
<td></td>
<td>49.4 ± 3.6</td>
<td>29.9 ± 3.2</td>
<td>52.0 ± 9.1</td>
</tr>
<tr>
<td>5 – 15</td>
<td></td>
<td>17.1 ± 3.2</td>
<td>10.2 ± 0.9</td>
<td>20.0 ± 2.8</td>
</tr>
</tbody>
</table>

a Zn at 22.5 kg/ha was applied as the sulfate source of Zn.
b Zn at 68 kg/ha was applied as the sulfate source of Zn.

At site 2 where Zn was applied at 22.5 kg/ha to the soil surface, the extractable and total Zn concentrations in the 2 to 5 cm soil depth increased after 575 mm of rain over 4 months (Table 5.3). Where 22.5 kg Zn as the sulfate was applied 4 months earlier, about 10 % of the applied Zn had moved into the 2-5 cm soil depth segment. Where Zn was applied at 68 kg/ha as the sulfate, the NH₄OX, ZnDTPA and total soil Zn concentration...
increased for the 5-15 cm soil depth. That is, about 12% of the applied Zn from the sulfate source had moved vertically into the 5.0-15 cm segment.

**Experiment 2**: Zinc from the oxide source always appeared to be in higher concentration in the surface 0-2 cm than the sulfate source (compare scintillation counts) (Table 5.4). However, after 374 days and 1438 mm of rain, $^{65}$Zn initially applied to the surface of the soil was detected in the 5-6 cm soil depth for the sulfate and in the 4-5 cm for the oxide source (Table 5.4). There was no detectable $^{65}$Zn from the insoluble oxide source in the 5-6 cm soil depth. The soluble Zn sulfate source was evenly distributed in the surface to 4 cm of soil, while Zn from the oxide was still concentrated in the top 2 cm of the surface soil (Table 5.4). At day 374, 95% of the sulfate and 99% of the oxide source of the Zn was accounted for in the top 4 cm of the soil (Table 5.4).

Table 5.4. Distribution of $^{65}$Zn applied either as a sulfate or oxide source, within a soil profile at different times since application; Experiment 2. The Zn was applied to the soil surface at 0.75 kg Zn/ha. Data are counts per 100 s corrected for isotope decay and background count.

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Days since $^{65}$Zn applied</th>
<th>Sulfate</th>
<th>Oxide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4</td>
<td>18</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>(25)$^{a}$</td>
<td>(96)</td>
<td>(194)</td>
</tr>
<tr>
<td>0 – 1</td>
<td>29121</td>
<td>21135</td>
<td>14678</td>
</tr>
<tr>
<td>1 - 2</td>
<td>2422</td>
<td>9933</td>
<td>14188</td>
</tr>
<tr>
<td>2 - 3</td>
<td>98</td>
<td>313</td>
<td>2655</td>
</tr>
<tr>
<td>3 - 4</td>
<td>b</td>
<td>93</td>
<td>2381</td>
</tr>
<tr>
<td>4 – 5</td>
<td></td>
<td>451</td>
<td>457</td>
</tr>
<tr>
<td>5 - 6</td>
<td></td>
<td>183</td>
<td>191</td>
</tr>
<tr>
<td>6 - 7</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^{a}$ Cumulative rainfall (mm) after the addition of radioactively labelled Zn fertiliser is in parentheses.

$^{b}$ No recorded reading indicates that no detectable Zn$^{65}$ was measured by scintillation count for 100s.

5.1.5 Discussion

There is little downward movement of Zn either applied as the soluble or insoluble source in soils of WA, even in soils of low clay content and low cation exchange capacity in high
rainfall districts. Similar to this study, various workers have found little or no movement of Zn either applied as insoluble, soluble or organic complexed (chelates) in a range of soil textures (Jones et al. 1957; Korte et al. 1976; Novillo et al. 2002; Obrador et al. 2003).

As in this study, Jones et al. (1957) found that Zn had moved to depths of 5 cm after 900 mm of rain when applied with copper sulfate and superphosphate, both of which increased the movement of Zn downward. Copper sulfate and superphosphate were applied in both our experiments; however, it cannot be determined if either superphosphate with its Zn contaminant or the copper sulfate contributed to the minimal Zn movement down the soil profile. The grey sand of this study had a higher cation exchange capacity (CEC) (4.1 cmol/kg compared with 1.2 cmol/kg) and appreciably higher organic carbon (1.4 % organic carbon compared with 0.2 % organic matter) than the sandy soils used by Jones et al. (1957) however, the movement of Zn in the soils was similar. In calcareous sand (CEC 5.7 cmol/kg and clay 3 %) there was little movement of Zn and additions of copper sulfate or superphosphate had no effect on movement of Zn down the soil profile (Jones et al. 1957). Although comparisons among various reported studies of Zn leaching are difficult to make, in most situations where levels of Zn applied are comparable to recommended fertiliser amounts there is little movement of Zn down the soil profile. In addition to CEC, the movement of Zn down the soil profile would be affected by those factors that influence Zn reactions, viz the organic carbon content, soil pH (Jones et al. 1957; Singh 1974; Singh and Shukla 1976) (see also Chapter 2–4) and initial soil Zn content (Singh 1974). As soil properties and basal fertilisers were not manipulated in this study, it was impossible to determine if these soil properties and addition of other nutrient elements affected the minimal downward movement of Zn in this soil.

The movement of Zn down the soil profiles after high amounts of Zn had been applied to
the soil surface (Table 5.3) is in agreement with studies of Nelson and Melsted (1955) and Jurinak and Thorne (1955). In this study, where Zn moved down the soil profile to depth, Zn sulfate were added at amounts that provided 22.4 to 68 kg Zn/ha. These additions of Zn (22.4–68 kg Zn/ha) are extremely high compared with the 0.75 to 1.5 kg Zn/ha generally used in cereal and pasture growing areas of WA (Gartrell and Glencross 1968). The movement of Zn at high levels of application may be due to the saturation of Zn adsorption sites of the soil. In the Indo-Gangetic plains of India where annual Zn applications are typically 10 kg Zn/ha, redistribution of Zn by leaching may be a significant issue in the cycling of Zn (Takkar et al. 1975; Takkar and Walker 1993). It may also have environmental implications if leached Zn reaches the groundwater. However, such risks seem very improbable under current Zn fertiliser practice in southwest Australia.

The movement of $^{65}$Zn (Table 5.4) compared with the movement of Zn determined by the extractants and total digestion (Tables 5.2 and 5.3) is of interest. The movement of $^{65}$Zn (sulfate source) from the surface to a depth of 5 to 6 cm (Experiment 2; Table 5.4), compared with the surface-applied sulfate (Experiment 1; Table 5.2) which was retained in the top 2.5 cm could possibly have been due to the time of application and the amount of rainfall immediately following the application of Zn fertiliser to the soil surface. The Zn sulfate applied in October received 80 mm of rain in the first month, while the $^{65}$Zn applied in June received 96 mm of rain in the first 18 days. The $^{65}$Zn applied in June received a total of 660 mm of rain over the following four months. In contrast, the Zn sulfate applied to the soil surface at site 1 in October received 170 mm of rain in the four months following the application of Zn. Such differences in the intensity and amount of rainfall in the period immediately after application before soil reactions have diminished plant available Zn may have caused the downward movement of Zn in the soil to differ.
The limited downward movement (immobility) of Zn in a sandy soil with low cation exchange capacity (4.0 cmol/kg) and low clay content (3.5 %) under high rainfall has important implications for the placement of Zn fertilisers in WA soils. The immobility of Zn may explain why surface applications of Zn fertilisers to soils have sometimes failed to correct Zn deficiency particularly in *P. radiata* (McGrath and Robson 1984). By contrast, in drier agricultural districts of WA, Zn fertiliser used for grain production of cereals is generally mixed with superphosphate and placed (drilled) with the seed when sowing the crop. This Zn probably remains in the shallow cultivated soil layer (0-10cm) in which most annual plants root freely and therefore can use the fertiliser Zn in this layer when the soil is moist. However, with comparable levels of application, very little of the Zn moved below 2.5 cm, and none below 5 cm which has implications for Zn fertilizer availability and placement for both pine and cereal production in WA (discussed further in Chapter 7).

*Applicability to losses of Zn from the soil-plant system of WA:* The data presented in this chapter shows that Zn applied to the surface of an acid sand followed by 1400 mm of rain only moved 5 cm vertically and 95 % of the applied Zn could be accounted for in this depth of soil. The results imply that the vertical movement of Zn in the drier regions of WA used for cereal production where the annual average rainfall is <500 mm is negligible. Moreover, the present results were obtained on an acid sand with extremely low clay content. Hence, on soils with higher clay content, Zn leaching would be even less probable. Therefore, losses of Zn below the root zone (>50 cm) by leaching are virtually non-existent. However, reactions between Zn and other soil properties could possibly lead to a decline in the availability of soil applied Zn. Reactions of Zn fertiliser added to soils and the measurement of decline in relative effectiveness are determined in the following sections of this Chapter.
5.2 Effect of soil properties on the relative effectiveness of applied Zn

5.2.1 Abstract

Chemical reactions of Zn with soil constituents are likely to decrease the residual effectiveness (RE) of Zn fertiliser for plant growth. In order to determine the significance of these reactions the effect of moist incubation on the availability of applied Zn for clover was examined in a glasshouse study using a range of Australian soils. On soils where plant growth was increased by Zn application, the prior incubation of Zn with the soil led to a decrease in plant growth response to the added Zn fertiliser relative to Zn applied immediately before (fresh Zn) sowing the clover seed. On all soils, incubating soil with applied Zn decreased the uptake of Zn by clover relative to freshly applied Zn. Similarly, the level of Zn in the soil measured by DTPA extractable Zn (Zn$_{\text{DTPA}}$) decreased where the Zn was incubated with the moist soil. The RE of Zn application for clover growth was measured by dry weight of shoots (RE$_{\text{DWS}}$), uptake of Zn by shoots (RE$_{\text{uptake}}$) and Zn$_{\text{DTPA}}$. Values obtained by all three methods of determining the RE were closely correlated.

The extent of the decline in availability of Zn with incubation differed among soils; being greater in alkaline soils, in soils with high clay content, in soils with high levels of organic carbon and in soils with free calcium carbonate. A multiple linear regression was used to explain the relationship between the RE for dry weight of shoots and soil properties ($R^2 > 0.90$):

\[ RE_{\text{DWS}} = 1.10 - 0.060 \text{pH}_{\text{Ca}} + 0.003 \text{ clay (\%)} - 0.018 \text{ organic carbon (\%)} + 0.017 \text{ calcium carbonate (\%)} \]

An earlier version was published a “Reaction of zinc with soil affecting its availability to subterranean clover. II. Effect of soil properties on the relative effectiveness of applied zinc”. RF Brennan (1990), Aust. J. Soil Res. 28, 303–310.
The concentration of Zn in youngest open blade (YOB) and in dried rest of shoots was used to determine critical concentrations for Zn in tissue. The relationship between Zn concentration in plant parts and the dry weight of shoots for both the freshly applied and incubated Zn gave a single response curve. The critical Zn concentration in the YOB, associated with 90% of the maximum relative yield of clover shoots, was 12 mg Zn/kg; the corresponding value for dried rest of shoots was 18-20 mg Zn/kg.

5.2.2 Introduction

Many Australian soils are naturally deficient in Zn as outlined in Chapter 3.1.2. In WA, Zn fertiliser is applied at amounts between 0.5 and 1.5 kg/ha and there is a decline in the effectiveness of fertiliser Zn with time over a range of conditions (Chapter 4.2).

Losses of Zn by leaching are negligible at application rates of 2 kg Zn/ha or less, even with high rainfall (1100mm annual average) on sands of WA (Chapter 5.1). Removal of Zn in farm products varies from 15 to 45 g/ha for pasture hay (J. Gartrell personal communication), 50 to 150 g/ha for cereal grain (Mengel and Kirby 1978) and 3 to 9 g/ha for wool (Masters and Somers 1980). Physical removal of soil by erosion would also result in loss of Zn from the soil-plant-animal system. All losses of Zn as a result of crop and animal product removal and soil loss would need to be considered in quantifying the decline in the residual effectiveness (RE) of Zn fertiliser in WA.

However, chemical reactions of Zn with soil constituents are likely to be especially important in decreasing the RE of fertiliser Zn. Reduction in the availability to wheat plants of applied copper by chemical and sorption reactions with soil has been demonstrated (Brennan et al. 1980, 1983, 1984) but changes in availability of applied Zn have been neither demonstrated nor measured.

In this work an incubation technique was used to determine how the availability of Zn to
plants declines with time and whether the rate of decline varies among soils of differing soil properties.

5.2.3 Materials and Methods

The experimental design and procedures have been outlined in a previous chapter (Chapter 3.1). Zinc applied before the warm moist incubation (about 30 °C) treatment is called incubated Zn. Zinc applied after the incubation treatment and immediately before sowing the clover seed is called fresh Zn. Soil numbers and soil properties are as for Chapter 2.1.

Statistical analyses: All data were analysed by standard analysis of variance where appropriate. Simple linear regression and stepwise multiple linear regression between RE, calculated by several methods, and soil properties were performed by standard statistical techniques as outlined in Chapter 2.1.3.

The relationships between added Zn and Zn content (the dry weight of shoots multiplied by the concentration of Zn in the plant parts, commonly called uptake) of clover shoots or Zn\textsubscript{DTPA} were adequately described by linear regression for each soil:

\[ Y = a + bx, \]

where \( Y \) is the Zn content of clover shoots (uptake) or DTPA Zn, \( x \) is the Zn applied to pots and \( b \) is the slope of the line. The Zn added before incubation and after incubation results in \( b_1 \) and \( b_F \), the slopes of the incubated (I) and freshly (F) applied Zn lines, respectively. The ratio of the two slopes (\( b_1/b_F \)) within each soil is the relative effectiveness (RE) of the fertiliser (Barrow and Campbell 1972; Brennan et al. 1980).

Similarly, for the relationship between added Zn and percentage maximum dry weight of shoots, the initial linear slopes were used following methods outlined in Bolland et al.
The ratio of the initial slopes of the incubated line \( b_I \) and the initial slope of the fresh \( b_F \) Zn application for the dry weight of shoots (DWS) \( b_I/b_F \) determines the REDWS of the Zn fertiliser. Hence in this study, the RE of the Zn application was determined by using the ratio of incubated to the freshly applied Zn in relation to: (i) the dry weight of shoots (REDWS), (ii) the content of Zn in shoots (REuptake) and (iii) DTPA soil extractable Zn (REDTPA).

**Critical Zn concentration in tissue:** The relationship between relative yield of dried whole shoots (yield of young tissue + yield of rest of shoots) and the concentration of Zn in either dried youngest open blades (YOB) or in dried whole shoots was used to define critical Zn concentrations in tissue. Mean data were fitted to the Mitscherlich equation by non-linear regression using a computer program written in compiler BASIC (Barrow and Mendoza 1990) as outlined in Chapter 2.1.3. Procedures for the determination of critical levels of Zn are also outlined in Chapter 2.1.3.

### 5.2.4 Results

**Experimental observations and plant analysis:** Symptoms of Zn deficiency in clover plants were readily recognised about 17 to 21 days after emergence of the seedlings as described in Chapter 2. However, in soils 9, 31, 38, 45 (Table 5.5) there were no visual symptom of Zn deficiency even though a 5 to 25 % yield increase to applied Zn was measured. Increases in DWS of clover to Zn fertiliser were not measured on several soils examined (11, 15, 17, 37, 41, 43, 44, 46, 53: Table 5.5).

For clover at flowering, Zn deficiency was associated with concentrations of Zn in the youngest open blades (YOB) of about 12 mg/kg (Figure 5.1a). The relationship between Zn concentration in rest of shoots and the dry weight of shoots across the range of soils tested was not as well defined as for the YOB (compare Figure 5.1a to Figure 5.1b).
Figure 5.1 The relationship between the percentage maximum dry matter production of clover (yield of dried young tissue + yield of the rest of shoots) and (a) the Zn concentration in the youngest open blade and (b) the Zn concentration in the rest of shoots of clover plants at 28 days after seeding. Maximum dry weight of shoots for 0.8 mg Zn/pot applied after the incubation treatment is equivalent to 100%. Soil No. (Table 5.5), Symbols soil 5 (-), soil 8 (●), soil 10 (◇), soil 13 (♦), soil 19 (●), soil 21 (+), soil 25 (○), soil 2 (x), soil 32 (○), soil 34 (□), soil 38 (Δ), soil 43 (▲).
Zinc deficiency was associated with concentrations of Zn in the rest of shoots of about 18 mg/kg (Figure 5.1b). By contrast, for the rest of shoot hand fitted curves and use of the Cate-Nelson procedure (Chapter 2.1) indicated a critical level of about 20 mg Zn/kg.

**Relative effectiveness estimated from dry matter production:**

<table>
<thead>
<tr>
<th>Soil No.</th>
<th>Relative Effectiveness&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Soil No.</th>
<th>Relative Effectiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zn&lt;sub&gt;app&lt;/sub&gt;</td>
<td>DWS&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Zn&lt;sup&gt;e&lt;/sup&gt;</td>
</tr>
<tr>
<td>1</td>
<td>0.68</td>
<td>0.65</td>
<td>0.65</td>
</tr>
<tr>
<td>2</td>
<td>0.75</td>
<td>0.82</td>
<td>0.79</td>
</tr>
<tr>
<td>3</td>
<td>0.74</td>
<td>0.72</td>
<td>0.74</td>
</tr>
<tr>
<td>4</td>
<td>0.80</td>
<td>0.83</td>
<td>0.77</td>
</tr>
<tr>
<td>5</td>
<td>0.78</td>
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<td>0.76</td>
</tr>
<tr>
<td>6</td>
<td>0.73</td>
<td>0.72</td>
<td>0.71</td>
</tr>
<tr>
<td>7</td>
<td>0.76</td>
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<td>0.75</td>
</tr>
<tr>
<td>8</td>
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<td>0.71</td>
</tr>
<tr>
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<td>0.69</td>
<td>0.73</td>
<td>0.74</td>
</tr>
<tr>
<td>10</td>
<td>0.75</td>
<td>0.75</td>
<td>0.78</td>
</tr>
<tr>
<td>11</td>
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<td>NR&lt;sup&gt;f&lt;/sup&gt;</td>
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</tr>
<tr>
<td>12</td>
<td>0.76</td>
<td>0.69</td>
<td>0.74</td>
</tr>
<tr>
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<td>0.75</td>
<td>0.74</td>
<td>0.69</td>
</tr>
<tr>
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<td>0.64</td>
<td>0.58</td>
<td>0.52</td>
</tr>
<tr>
<td>15</td>
<td>0.65</td>
<td>NR</td>
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</tr>
<tr>
<td>16</td>
<td>0.76</td>
<td>0.71</td>
<td>0.66</td>
</tr>
<tr>
<td>17</td>
<td>0.72</td>
<td>NR</td>
<td>0.67</td>
</tr>
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<td>0.76</td>
<td>0.65</td>
<td>0.75</td>
</tr>
<tr>
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<td>0.70</td>
<td>0.69</td>
</tr>
<tr>
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<td>0.73</td>
<td>0.76</td>
<td>0.74</td>
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<td>0.75</td>
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</tr>
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<td>0.63</td>
<td>0.63</td>
</tr>
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<td>0.54</td>
<td>0.52</td>
</tr>
<tr>
<td>25</td>
<td>0.62</td>
<td>0.62</td>
<td>0.60</td>
</tr>
<tr>
<td>26</td>
<td>0.61</td>
<td>0.57</td>
<td>0.60</td>
</tr>
<tr>
<td>27</td>
<td>0.71</td>
<td>0.72</td>
<td>0.69</td>
</tr>
</tbody>
</table>

<sup>a</sup>Relative effectiveness (RE) of the Zn application was determined by calculation of the ratio of the slope (b coefficient) of the incubated and freshly applied Zn; for DWS, initial slope of c coefficient for 3 methods.

<sup>b</sup>Soil No is the soil number as for Chapter 2.1.3.

<sup>c</sup>RE determined by zinc uptake.

<sup>d</sup>RE determined from dry weight of shoots.

<sup>e</sup>RE determined from DTPA Zn.

<sup>f</sup>No response to Zn application in dry weight of shoots.

Incubation of Zn fertiliser in moist soil decreased the DWS of clover for all 46 soils on which growth of clover was increased by Zn fertiliser. The relationship between DWS and Zn applied, either before or after incubation, resulted in a range of values for the RE
which varied greatly with soil types (Table 5.5).

In an attempt to group similar RE values together, the soil types were sorted on soil pH\textsubscript{Ca} values and on clay content (Table 5.6, Figure 5.2, and Figure 5.3). However, this sorting of soil types still resulted in a large range of RE\textsubscript{DWS} values for each particular soil classification (Table 5.6). For example, the acid sands (pH <7; Clay <8 %) had RE\textsubscript{DWS} values that ranged from 0.63 to 0.85, while for the alkaline sands the RE\textsubscript{DWS} values ranged from 0.54 to 0.68 (Table 5.6). Therefore, across the range of soils the RE\textsubscript{DWS} was related by regression to several soil properties.

Table 5.6. The range and mean of the relative effectiveness (RE) of incubated fertiliser Zn for clover grown on a range of Australian soils classified on the basis of soil pH\textsubscript{Ca} and clay content (%).

<table>
<thead>
<tr>
<th>Soil type</th>
<th>RE of Zn determined by:</th>
<th>Dry weight of shoots</th>
<th>Zinc content of shoots</th>
<th>DTPA extraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Acid sands\textsuperscript{a}</td>
<td></td>
<td>0.63 - 0.85</td>
<td>0.72</td>
<td>0.72 - 0.80</td>
</tr>
<tr>
<td>Alkaline sands\textsuperscript{a}</td>
<td></td>
<td>0.54 – 0.68</td>
<td>0.60</td>
<td>0.55 – 0.69</td>
</tr>
<tr>
<td>Acid sandy loams\textsuperscript{b}</td>
<td></td>
<td>0.48 – 0.79</td>
<td>0.64</td>
<td>0.60 – 0.73</td>
</tr>
<tr>
<td>Alkaline sandy loams\textsuperscript{b}</td>
<td></td>
<td>0.57 – 0.64</td>
<td>0.61</td>
<td>0.53 – 0.63</td>
</tr>
<tr>
<td>Alkaline clays\textsuperscript{c}</td>
<td></td>
<td>0.49 – 0.60</td>
<td>0.53</td>
<td>0.47 – 0.57</td>
</tr>
</tbody>
</table>

\textsuperscript{a} pH\textsubscript{Ca} < 7 acid; pH\textsubscript{Ca} > 7 alkaline.  \textsuperscript{b} sands < 8 % clay; sandy loam, < 25 % clay; \textsuperscript{c} Clays, > 25 % clay.

The simple relationship between the RE\textsubscript{DWS} and pH\textsubscript{Ca} explained 59 % of the variation (Figure 5.2) while the simple linear regression between RE\textsubscript{DWS} and clay content explained 42 % of the variation. However, the relationship improved (statistically significant) in a multiple regression with the inclusion of other soil properties (clay, organic carbon, free calcium carbonate content) (Table 5.7).

For the multiple linear regression, a good relationship ($R^2=0.904$) was obtained between four soil properties and the RE\textsubscript{DWS}. The four properties in decreasing order of importance were soil pH\textsubscript{Ca}, clay (%), organic carbon %, and free calcium carbonate (%). The selected
model for the RE of Zn determined by dry weight of shoots (Table 5.8) was:

\[ RE_{DWS} = 1.10 - 0.060 \text{pH}_{Ca} + 0.003 \text{ clay (\%)} - 0.019 \text{ organic carbon (\%)} + 0.016 \text{ calcium carbonate (\%)} \]

Relative effectiveness estimated from zinc content of shoots and DTPA extractable Zn: For all 54 soils, where Zn was applied, incubation of moist soil depressed the content of Zn (uptake) in shoots of clover and extractable Zn\textsubscript{DTPA}. The decrease in Zn uptake and the depression in Zn\textsubscript{DTPA} values resulted in a range of RE values determined for both uptake and Zn\textsubscript{DTPA}, which varied with soil type (Table 5.6).

Within each soil class (e.g. acid sand, alkaline clays) there was still a great range in RE values determined by uptake and Zn\textsubscript{DTPA} (Table 5.6). For example, in the acid sands,
measured $RE_{\text{uptake}}$ values ranged from 0.72 – 0.80 while for alkaline sands the $RE_{\text{uptake}}$ values ranged from 0.55 to 0.69 (Table 5.6). Similarly, the $RE_{\text{DTPA}}$ for the acid sands ranged from 0.80 to 0.9, while the $RE_{\text{DTPA}}$ for the alkaline sands ranged from 0.52 to 0.72 (Table 5.6).

![Graph showing the relationship between RE uptake and soil pH Ca and clay content.](image)

**Figure 5.3** The simple linear regression between RE of incubated Zn fertiliser for dry weight of clover shoots and the clay content of soil.

The RE of applied Zn either measured by plant uptake or $Zn_{\text{DTPA}}$ was closely related by simple linear regression to soil pH$_{Ca}$ and to the clay content (Table 5.7). The linear relationship between $RE_{\text{uptake}}$ and soil pH$_{Ca}$ explained 74 % of the variation (Figure 5.4). Similarly, the linear relationship between $RE_{\text{uptake}}$ and clay content explained 65 % of the variation (Figure 5.5).

For the multiple linear regression, there was a marked statistical (significant) improvement in the relationship between $RE_{\text{uptake}}$ and measured soil properties of pH$_{Ca}$, clay content, organic carbon and free calcium carbonate that accounted for 90 % of the variation (Table 5.7). The selected model (see Table 5.8) for RE calculated from uptake

$$y = -0.004x + 0.7117$$

$$R^2 = 0.42$$

This model equation is used to predict the RE for dry weight of shoots based on the clay content (%).

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was:

\[ \text{RE}_{\text{uptake}} = 1.047 - 0.055 \, \text{pH}_{\text{Ca}} - 0.004 \, \text{clay} \, (\%) - 0.011 \, \text{organic carbon} \, (\%) + 0.015 \, \text{calcium carbonate} \, (\%). \]

**Table 5.7.** The statistical relationship amongst soil properties and the RE of incubated Zn fertilizer determined from dry weight of clover shoots, Zn uptake or DTPA-Zn for a range of Australian soils.

<table>
<thead>
<tr>
<th>Dependent variable</th>
<th>Independent variable</th>
<th>Simple regression</th>
<th>Multiple linear regression</th>
</tr>
</thead>
</table>
| RE of Zn for clover growth | Aluminium oxide | 0.049 ns | 0.911 a^
| | Iron oxide | 0.143 ns \(b\) | 0.911 a |
| | DTPA Zn | 0.389 \(*\) | 0.909 a |
| (n =46)\(c\) | Calcium carbonate | 0.234 ns | 0.904 a |
| | Organic carbon | 0.072 ns | 0.869 b |
| | Clay | 0.546 \(*\) | 0.832 c |
| | pH\(_{\text{Ca}}\) | 0.591 ** | 0.591 d |
| RE of Zn for iron oxide | Aluminium oxide | 0.096 ns | 0.907 \(\Lambda\) a |
| | Iron oxide | 0.106 ns | 0.906 a |
| RE of Zn for DTPA Zn uptake (n=54) | DTPA Zn | 0.063 ns | 0.906 a |
| | Organic carbon | 0.016 ns | 0.904 a |
| | Calcium carbonate | 0.208 ns | 0.888 b |
| | Clay | 0.642 \(*\) | 0.846 c |
| | pH\(_{\text{Ca}}\) | 0.755 ** | 0.755 d |
| RE of Zn for DTPA Zn extractable (n=54) | DTPA Zn | 0.024 ns | 0.888 a |
| | Iron oxide | 0.188 ns | 0.881 a |
| | Organic carbon | 0.107 ns | 0.871 a |
| | Calcium carbonate | 0.057 ns | 0.739 b |
| | Clay | 0.443 \(*\) | 0.604 c |
| | pH\(_{\text{Ca}}\) | 0.549 ** | 0.549 d |

\(a\) The \(R^2\) value is for the regression including the respective variable and those following it. In the multiple regression column \(R^2\) values followed by the same letter are not significantly different.

\(b\) Significant simple linear regression relationships \(*\) \((P<0.05)\) \(**\) \((P<0.01)\), ns not significant.

\(c\) \(n\) = the number of soils in the regression; note, there were yield increases to Zn application in only 46 soils; 8 soils were non-responsive.

The simple linear relationship between \(\text{RE}_{\text{DTPA}}\) and soil pH\(_{\text{Ca}}\) explained 53 % of the variation in the measured RE values (Figure 5.6). The selected model for RE calculated from the depression in Zn\(_{\text{DTPA}}\) as a result of the incubation treatment was:
$RE_{DTPA} = 1.049 - 0.048 \, pH_{Ca} - 0.004 \, clay\, (\%) - 0.032 \, organic\, carbon\, \% + 0.028 \, calcium\, carbonate\, (\%)$.

**Figure 5.4** The relationship between soil $pH_{Ca}$ for a range of soils and the relative effectiveness of Zn as measured by Zn content of clover shoots.

**Figure 5.5** The relationship between clay content of soils and the relative effectiveness of Zn as measured by Zn content of clover shoots ($RE_{uptake}$).
This model for $R_{EDTPA}$ accounted for 87% of the variation (Tables 5.7 and 5.8).

The relationship between the $R_{EDTPA}$ and soil properties was improved by the same soil properties as $R_{Euptake}$ (Table 5.7).

![Figure 5.6](image)

Figure 5.6 The relationship between the relative effectiveness of incubated Zn as measured by DTPA extractable Zn and soil $pH_{Ca}$.

**Relationships for soils of WA:** The WA soils selected in this study had little or no free calcium carbonate; hence this soil property was not considered for the following relationships:

(i) \[ R_{EDWS} = 1.04 - 0.058 \, pH_{Ca} - 0.002 \, \text{Clay} \% - 0.016 \, \text{organic carbon} \% \]

(ii) \[ R_{Euptake} = 1.004 - 0.052 \, pH_{Ca} - 0.003 \, \text{Clay} \% - 0.009 \, \text{organic carbon} \% \]

(iii) \[ R_{EDTPA} = 0.964 - 0.04 \, pH_{Ca} - 0.002 \, \text{Clay} \% - 0.026 \, \text{organic carbon} \% \]

These models for RE accounted for about 70% of the variation found in RE values for WA soils.
Table 5.8. Multiple linear regression models for relative effectiveness (RE) of fertiliser Zn, where RE are based on dry weight of shoots (DWS) of clover shoots, Zn uptake of clover and soil DTPA extractable Zn. Cross reference to Table 5.7.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Coefficient</th>
<th>SE</th>
<th>t-statistic</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>RE of Zn for clover growth (n =46)³</td>
<td>pH Ca</td>
<td>-0.060</td>
<td>0.005</td>
<td>-10.935</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>0.003</td>
<td>0.0004</td>
<td>-6.438</td>
</tr>
<tr>
<td></td>
<td>Organic carbon</td>
<td>-0.019</td>
<td>0.004</td>
<td>-4.750</td>
</tr>
<tr>
<td></td>
<td>Calcium carbonate</td>
<td>0.016</td>
<td>0.0039</td>
<td>4.050</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>1.103</td>
<td>0.1422</td>
<td>0.90</td>
</tr>
<tr>
<td>RE of Zn for clover uptake (n=54)³</td>
<td>pH Ca</td>
<td>-0.055</td>
<td>0.051</td>
<td>-10.756</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>-0.004</td>
<td>0.0038</td>
<td>-9.055</td>
</tr>
<tr>
<td></td>
<td>Organic carbon</td>
<td>-0.011</td>
<td>0.0038</td>
<td>-2.985⁴</td>
</tr>
<tr>
<td></td>
<td>Calcium carbonate</td>
<td>0.015</td>
<td>0.0028</td>
<td>5.279</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>1.047</td>
<td>0.1309</td>
<td>0.90</td>
</tr>
<tr>
<td>RE of Zn for DTPA extract Zn (n=54)³</td>
<td>pH Ca</td>
<td>-0.048</td>
<td>0.0059</td>
<td>-8.154</td>
</tr>
<tr>
<td></td>
<td>Clay</td>
<td>-0.004</td>
<td>0.0004</td>
<td>-9.339</td>
</tr>
<tr>
<td></td>
<td>Organic carbon</td>
<td>-0.032</td>
<td>0.0044</td>
<td>-7.399</td>
</tr>
<tr>
<td></td>
<td>Calcium carbonate</td>
<td>0.028</td>
<td>0.0032</td>
<td>8.635</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>1.049</td>
<td>0.1312</td>
<td></td>
</tr>
</tbody>
</table>

³ Standard error.
⁴ Statistically significant at the P<0.001 probability level except one level indicated below (footnote ⁴).
³ n = the number of soils; note, there were yield increases to Zn application on only 46 soils; 8 soils were non-responsive.
⁴ P=0.004.

Comparison of the methods of determining the relative effectiveness: All methods were closely associated and there was a good correlation between all 3 methods (Figure 5.7). This suggests that for non-responsive soils, Zn content of plant shoots can be used for the determination of RE. In following chapters, both the use of Zn content of plant shoots and yield are used for these calculations to determine RE of Zn.

5.2.5 Discussion

The incubation of Zn with moist soil for 30 days at 30°C decreased its availability for uptake by roots of clover. A decrease in dry weight of clover shoots and the Zn content of clover shoots quantified the effect of the incubation of Zn with soil on its availability. The effect of the moist incubation of Zn with the soil was reflected also in the decreased amount of Zn extracted by DTPA for all soil types. This reduction in the effectiveness of
applied Zn is interpreted as evidence of soil reactions converting a proportion of the applied Zn into forms unavailable for uptake by roots of plants.

![Graph showing the relationship between the relative effectiveness determined by Zn uptake and the relative effectiveness determined by dry weight of shoots and the relative effectiveness (RE) determined by DTPA Zn. Symbols: RE for dry weight of shoots (●); RE for DTPA Zn (■).](image)

**Figure 5.7** The relationship between the relative effectiveness determined by Zn uptake and the relative effectiveness determined by dry weight of shoots and the relative effectiveness (RE) determined by DTPA Zn. Symbols: RE for dry weight of shoots (●); RE for DTPA Zn (■).

The decline in the availability of Zn could not be specifically related to any one-soil property, although soil pH and/or clay content accounted for about 50% of the variation determined by the three methods of this study. However, the multiple linear regression analysis between the RE of Zn and soil properties showed that pH<sub>Ca</sub>, clay (%), organic carbon (%) and free calcium carbonate (%) were important soil properties. These soil properties explained about 85% of the variation in RE regardless of whether RE was assessed by shoot dry weight, shoot Zn uptake or Zn<sub>DTPA</sub>. Although there appears to be no literature available for the residual effectiveness as defined and measured in this study,
the determined soil properties for the multiple linear regressions also appear to be important properties of soils affecting the availability of Zn to plants. For example, Martens (1968) found that the soil properties of soil pH, organic matter and clay were important in determining the uptake of Zn by corn plants.

Calcium carbonate and soil pH are important soil properties determining the availability of Zn to plants (Lucas and Knezek 1972; McGregor et al. 1974). The addition of calcium carbonate to an acid soil decreased the availability of Zn for plant uptake by soil adsorption. However, in alkaline calcareous soils the effect of soil pH in controlling the availability of Zn to plants may be through precipitation of Zn compounds of lower solubility (Clark and Graham 1968; Saeed and Fox 1977) or by the adsorption of Zn by carbonates (Udo et al. 1970). Agricultural production has acidified WA soils that were initially neutral to marginally acidic when first cleared of native vegetation for agriculture. This has increased the concentration of soluble aluminium (Al) in soil solution to toxic levels for plant roots thereby reducing root growth and grain yields of crops (Dolling et al. 1991, 1994; Dolling and Porter 1994). Soil acidification increases the availability of Zn in soil. The acidity problem is now being ameliorated by applications of lime and dolomite which increases soil pH, reduces Al toxicity and decreases the availability of Zn in soil to plants. When Zn supply in soil is marginal for crop production, liming the soil has induced a deficiency (Lucas and Knezek 1972). Few soils of WA have free calcium carbonate. Hence, calcium carbonate could be omitted from the multiple regression equations determined in this chapter since the effect of added lime on acid soils would be reflected in the soil pH value.

The role of organic matter in binding Zn has been extensively reviewed (Follet and Lindsay 1970; Martens et al. 1966; Stevenson and Ardakani 1972). The association of Zn with soluble and insoluble organic matter complexes (Hodgson et al. 1965, 1966;
Stevenson and Ardakani 1972) suggests that these forms of Zn in the soil decrease the availability of soil Zn for plant growth and decreases the relative effectiveness of applied Zn. Brennan et al. (1983) found that organic matter reduced the relative effectiveness of incubated copper, and it would appear that organic matter and its range of organic complexes are involved in the reactions of Zn with soil although the specific role of organic matter was not investigated in this chapter.

Zinc is adsorbed by clays (Reddy and Perkins 1974; Farrah and Pickering 1976, 1978; Farrah et al. 1980); however, the effects of the adsorption reactions on the availability of applied Zn to plants are not well researched. The percentage clay is an important soil property in determining the uptake of Zn by plants. Martens (1968) has found that as the clay content increased the availability of Zn declined, possibly through Zn sorption on clay constituents. This present work suggested that the reaction of Zn with clay is also important in determining the effectiveness of Zn incubated in the soil compared to freshly applied Zn.

The soil properties of soil pH, clay content, organic carbon and calcium carbonate content have been shown to affect both the initial effectiveness of Zn to plants and the residual effectiveness of incubated Zn. Therefore, the present work has provided a method of differentiating between soils in their potential for reducing the residual effectiveness of Zn fertiliser and this could be used to identify field situations most likely to require repeat applications of Zn to maintain maximum growth of clover. The initial effectiveness and residual effectiveness of Zn fertiliser is likely to be lower in alkaline calcareous soils compared to the neutral to acid sandy soils that represent about 75 % of the soils in the agricultural areas of WA (McArthur 1991). Although the incubation technique has identified soils likely to require more frequent repeat applications (that is, the residual value declines more rapidly compared to other soils), field studies are required to test the residual effectiveness in the field. Field studies are essential as there are additional
variables that might affect Zn availability including: wetting/dying cycles of the soil profile, crop species and cycling of Zn in the plant to soil system. The inclusion of pastures and grazing animals in the crop rotation may also have a bearing on Zn residual effectiveness. The following chapter examines some of the aspects of the response of crop species to both fresh and previously applied Zn in glasshouse and field studies.
Chapter 6

Responses of Plant Species to Zinc Fertiliser

6.1 Comparing Zn requirements of *Lupinus angustifolius* and *Lupinus luteus* for seed production.

6.1.1 Abstract

*Lupinus angustifolius* has been developed for sandy acidic soils to improve soil fertility and act as a break crop for controlling diseases and pests of cereals. *Lupinus luteus* is being assessed as a possible alternative grain legume to *L. angustifolius* in WA. The Zn requirement of *L. luteus* is not known; while that of *L. angustifolius* has only been measured in one previous field experiment in WA. In the same experiment used to measure the residual value (RV) of Zn for wheat in 1996 (Chapter 4.2), in 1997 the RV of Zn for three different lupins (*L. angustifolius* cv. Gungurru, and *L. luteus* cvv. Motiv and Teo) was measured using grain yield. The RV for the lupins was measured for Zn applied once only, either in 1997 (current Zn) or in a previous year. The previous Zn was applied in one of the following years to plots not treated with Zn before: 1983, 1984, 1986, 1990, and 1992. Both currently and previously applied Zn fertiliser increased lupin seed yields. When no Zn was applied, *L. luteus* produced larger grain yields than *L. angustifolius*. For each lupin species, the effectiveness of previous Zn relative to the effectiveness of current Zn decreased with increasing time since application of the Zn fertiliser for all three lupins. The decrease in RV was larger for *L. luteus* cv. Teo but was similar for *L. angustifolius* cv. Gungurru and *L. luteus* cv. Motiv.

An earlier version of this Chapter was first published as “Comparing how *Lupinus angustifolius* and *Lupinus luteus* use zinc fertiliser for seed production”. RF Brennan, MDA Bolland, G Shea (2001), *Nutrient Cycling in Agroecosystems*, 59, 209-21.
Fourteen years after application, the decrease in RV was 96 % for *L. luteus* cv. Teo compared with about 65 % for the other two lupins. For Zn applied in the current year, relative to *L. angustifolius* cv. Gungurru, *L. luteus* cv. Motiv used Zn about 45 % less effectively and so required about twice as much Zn to produce the same percentage of the maximum grain yield. *Lupinus luteus* cv. Teo used Zn about 70 % more effectively than *L. angustifolius* cv. Gungurru so only required about two thirds the Zn needed by *L. angustifolius* to produce the same relative yield. For Zn applied in each of the previous years, and relative to *L. angustifolius* cv. Gungurru in each of those years, *L. luteus* cv. Motiv used previously applied Zn from about 3 to 33 % more effectively for producing grain, whereas *L. luteus* cv. Motiv used previously applied Zn about 15 to 88 % less effectively.

6.1.2 Introduction

*Lupinus angustifolius* has been developed for sandy acidic soils in WA (Gladstones 1990): these soils comprise about 75 % of the approximate 18 million hectares used for agriculture in WA. *Lupinus angustifolius* is grown to improve soil fertility and act as a break crop for controlling diseases and pests of cereals, as well as producing seed (grain) for income (Hamblin 1987, Rowland *et al.* 1988, Delane *et al.* 1989). However, as *L. angustifolius* has been more widely grown it has become increasingly affected by several diseases which *L. luteus* appears to tolerate (Sweetingham *et al.* 1996). In addition, *L. luteus* is better adapted than *L. angustifolius* to very acidic soils (Foy 1997). As the sandy soils of WA have become more acidic, aluminium (Al) toxicity has become a problem (Dolling *et al.* 1994). *L. luteus* appears to tolerate Al toxicity better than *L. angustifolius* (Sweetingham *et al.* 1996). Consequently, *L. luteus* is being assessed as a possible alternative grain legume to *L. angustifolius* in WA.

When first cleared for agriculture, most of the WA wheatbelt soils were acutely deficient
in phosphorus (P), so profitable agricultural production was only achieved by applying fertiliser P as single superphosphate (superphosphate) that was Zn-contaminated (Bolland 1998). In addition to the Zn incidentally applied in the superphosphate, many farmers in WA have also applied Zn fertiliser directly to the soil as ZnO. In such cases where superphosphate was applied the initial application of Zn has been sufficient to meet the Zn requirements of wheat for many years (Anon. 1961; Ozanne et al. 1965: Chapter 4.2); particularly where at least 150 kg/ha of superphosphate (Zn-contaminated) was applied annually thereafter (Chapter 4.2).

Since annual superphosphate applications are less common, and superphosphate in WA is increasingly being manufactured from rock phosphate with lower Zn impurities, deficiencies of Zn are now being observed in many wheat growing areas (Brennan 1998, 2000). The increased usage of DAP fertiliser, which contains about one-twelfth of the amount of Zn found in superphosphate made in WA, has often resulted in immediate Zn deficiency in wheat (Brennan 1998) and *L. angustifolius* (Riley et al. 1992).

The Zn requirement of *L. luteus* grown on acidic soils is not known, while the Zn requirement of *L. angustifolius* have been studied in only one field experiment in WA (Riley et al. 1992). Farmers need to know the length of time that Zn supply in contaminated Zn in superphosphate or as ZnO applied in previous years remains fully effective for profitable grain production of lupin crops.

This chapter reports the results of a long-term field experiment conducted to assess whether the original applications of Zn to the soil up to 14 years before 1997, either as Zn-contaminated superphosphate or as ZnO applied with superphosphate, or as ZnO previously applied with DAP were still sufficient for *L. angustifolius* and *L. luteus* to produce profitable grain yield in 1997. This experimental work compared the effectiveness of fertiliser Zn for grain production. The same long-term field experiment
used to measure the RV of Zn for wheat production was re-used in 1997 to measure the RV of Zn for lupins and the results are reported in this section. In addition, the experiment compared how effectively each lupin species used previously applied and current Zn. Preliminary experiments have shown that seed of *L. luteus* has higher concentrations of cadmium (Cd) than grain of *L. angustifolius*, the experiment reported here was used to extend this comparison and to examine if there was a relationship between Zn and Cd concentration in the grain.

### 6.1.3 Materials and Methods

**Soil and Site:** Details of the location, climate and history of the field experiment from 1983 to 1996 are provided in Chapter 4.2.3. The experiment was used in 1997 for the experiment reported here, and details for 1997 are now provided.

**Experimental procedures in 1997:** In 1997, the following three lupins were sown in separate 1.4 by 50 m plots for each previous or current Zn treatment: *L. angustifolius* cv. Gungurru and *L. luteus* cvv Motiv and Teo. Eight rows of seeds, 175 mm apart, were sown along each plot in mid-May. Seeds of *L. angustifolius* and *L. luteus* were inoculated with *Bradyrhizobium lupini* strain WU 425 and lime-pelleted immediately before sowing the seed at 120 kg/ha.

Basal fertilisers applied to all plots to ensure that Zn was the only nutrient element limiting lupin yield were: (1) CoSO$_4$.7H$_2$O (0.25 kg/ha; 22 % Co), Na$_2$BO$_4$ (3.0 kg/ha; 8 % B), applied to the soil surface immediately before sowing; (2) CuSO$_4$.5H$_2$O (6.0 kg/ha; 25 % Cu) and NaMoO$_4$.2H$_2$O (0.25 kg/ha; 39 % Mo) were drilled with the seed (3) KCl (100 kg/ha; 50 % K) and gypsum (150 kg/ha; 17 % S) applied to the soil surface 4 weeks after emergence of the seedlings.

All weeds were successfully controlled using pre- and post-emergent herbicides. Insects,
red legged earth mite (*Halotydeus destructor* Tucker), aphids (*Acyrthosiphon kondoi* Shinji) and native bud worm (*Heliothis punctiger* Wallengren) were controlled with pesticides as required.

**Measurements:** Lupin seedlings at the 2 to 3 leaf stage, about 28 days after sowing, were counted along 1-m lengths of two adjacent rows at five random positions within each plot. In order to avoid contamination with Zn from the harvesting machine, 20 to 25 plants were collected at random locations within the middle six rows of each plot. Replicate samples of grain were milled and digested, and the Zn concentration in the digest was analysed by atomic absorption spectrophotometry.

Grain yield was measured by machine harvesting grain from the middle six rows of each plot in late November-early December and weighed. Sub-samples of grain were milled and analysed for concentration of nutrient elements other than Zn. To determine P, samples were digested in sulfuric acid and hydrogen peroxide (Yuen and Pollard 1954). P concentration in the digest was determined colorimetrically by the vanado-molybdate method. For Cd, samples were digested in nitric-perchloric acid mixture (McQuaker *et al.* 1979) and the concentration of Cd measured using inductively coupled plasma-atomic emission spectrometry.

**Analysis of Data:** The relationship between grain yield and the amount of Zn applied was fitted to a Mitscherlich equation of Barrow and Mendoza (1990):

\[
y = a - b \exp(-cx)
\]

where *y* is the grain yield (kg/ha), *x* is the amount of Zn applied (kg Zn/ha) and *a*, *b*, and *c* are coefficients. Coefficient *a* provides an estimate of the asymptote or maximum yield plateau. Coefficient *b* (kg/ha) estimates the difference between the asymptote and the intercept on the yield axis at *x* = zero and so indicates the maximum increase in grain
yields due to the application of Zn fertiliser. Coefficient $c$ (ha/kg Zn) describes the shape of the relationship and governs the rate at which $y$ (the yield response) increases as $x$ (the amount of Zn applied) increases (Ratkowsky 1990). Mean data were fitted to the equation by non-linear regression using a computer program written in compiler BASIC (Barrow and Mendoza 1990). The simplex method (Nelder and Mead 1965) was used to locate the least squares estimate of the non-linear coefficients.

As the three different lupins produced significantly different grain yields for the nil-Zn treatment and different maximum yield plateaux, it is not valid to use the $c$ coefficient of the Mitscherlich equation to compare yield responses of the species to applied Zn fertiliser (Barrow and Campbell 1972, Barrow 1985). Instead the initial slope of the relationship between grain yield and the amount of Zn applied was used to compare the yield response of the species to Zn application. For the Mitscherlich equation, as $x$ approaches zero, $\frac{dy}{dx}$ tends to $bc$ so $bc$ was used as an estimate of the initial slope (Barrow and Campbell 1972, Barrow 1975).

For grain production of each lupin species, the effectiveness of Zn applied in each of the previous years was calculated relative to the effectiveness of Zn applied in the current year (1997), to provide relative effectiveness ($RE_{Zn}$) values. For each lupin species, this was done by dividing $bc$ values of Zn applied in the current year (1997) or each of the previous years (1983, ’84, ’86, ’90 and ’92) by $bc$ for Zn applied in the current year (1997); therefore, by definition, for each lupin species, the $RE_{Zn}$ for Zn applied in the current year is always 1.00.

Then the effectiveness with which each of the three lupins used Zn applied in the current year or in each of the previous years was compared. For Zn applied in each of these years, this was done by dividing $bc$ of each lupin by $bc$ for $L. angustifolius$ cv. Gungurru, so by definition, the $RR_{species}$ for $L. angustifolius$ cv. Gungurru is always 1.00, regardless
of the year in which the Zn was applied. Comparisons were made relative to *L. angustifolius* because it is the major grain legume grown in WA and because *L. luteus* is being researched as a possible alternative grain-legume crop.

Simple linear regression by a standard computer program was used to examine the relationships between the concentration of Cd in seed, as the dependent variable (y axis), and the concentration of Zn in the seed, as the independent variable (x axis). The equation used was:

\[ y = A + Bx, \]

where *y* is the concentration of Cd (mg/kg) in the seed, and *x* is the concentration of Zn (mg/kg) in the seed. The coefficient of determination \( R^2 \) was determined for the relationship.

The relationship between grain yield and Zn concentration in the grain was used to determine the critical concentration in the grain as outlined in Chapter 2.

### 6.1.4 Results

Analysis of variance indicated highly significant \( (p < 0.001) \) effects on grain yield due to levels of Zn addition and significant \( (p< 0.05) \) effects due to year of application and the interaction of the amount of Zn applied by year of application.

**Plant density**: Additions of fertiliser Zn in the current or previous years did not affect the plant emergence. Plant densities (plant/m\(^2\)) did not differ for the different species (standard errors in brackets): *L. angustifolius* 48 (8); *L luteus* cv. Motiv 49 (9); and *L. luteus* cv. Teo 52 (5). These densities are adequate for maximum lupin grain production of both species in WA (Nelson and Delane 1990).

**Zn deficiency symptoms**: Symptoms of Zn deficiency, as brown spots on younger leaves,
was observed on all three lupins, but was much more obvious on *L. angustifolius* cv. Gungurru than the two *L. luteus* cultivars. The symptoms for *L. angustifolius* occurred for plants grown without added Zn (nil Zn) and those plots with low amounts of Zn applied 9 and 14 years previously. The symptoms of Zn deficiency for *L. angustifolius* observed in this study are in agreement with Snowball and Robson (1986). Deficiency symptoms for *L. luteus* cv. Motiv and Teo only occurred on the nil Zn treatments. At the start of flowering (early September), many of the *L. angustifolius* plants on the nil Zn treatment plots had died due to Zn deficiency. By contrast, only a few plants of *L. luteus* had died on the nil-Zn plots.

**Grain Yield:**

(a) *Zinc as superphosphate:* There were 2 superphosphate treatments. In one, superphosphate was applied to supply the same amount of P applied as that applied with DAP treatments. The second superphosphate was the same, except 1.5 kg Zn as ZnO was applied in 1983 only. In both superphosphate treatments, about 90 g Zn/ha was applied annually as Zn present in the superphosphate. In 1997, both the superphosphate treatments produced lupin grain yields on the maximum yield plateau achieved for ZnO treatments applied in the current year (1997). Evidently, the two superphosphate treatments provided sufficient Zn for grain production of all three lupins in 1997.

(b) *Zinc applied as ZnO:* For simplicity and clarity, data are only shown for the grain yield increase to Zn applied in three years, viz 1983, 1985 and 1997 (Figure 6.1). All other years fitted the same pattern of response. Current and previously applied Zn increased yield of *L. angustifolius* and *L. luteus* (Figure 6.1) although a higher amount of Zn previously applied was required to reach the same grain yield. For example, about 3 kg Zn/ha applied in 1983 was required for maximum grain yield of Gungurru (Fig 6.1 a) and Teo (Figure 6.1 b) while about 1.5 kg Zn/ha was required for Gungurru and about 1.0
kg Zn/ha for Teo applied in the current year.

(c) Zinc oxide effectiveness in current and previous years (RE_{Zn} values): The reason that the Zn applied in 1983 appears to have a sigmodial relationship to yield (Fig 6.1a; 6.1c) is probably because the Zn in this treatment had been in contact with the soil for the longest period. There was therefore more time for the slow reactions between the soil and Zn to occur which has been shown to decrease the effectiveness of Zn added to soil (Barrow 1987; Chapter 3 & 5.2). In addition, the Zn applied in previous years was increasingly incorporated throughout the soil when sowing crops with tined machines, which may increase retention of Zn by the soil. Thoroughly incorporated Zn fertiliser is then in contact with a greater soil volume allowing chemical reactions to take place more extensively and further diminish the availability of the applied Zn for plant uptake. Such reactions have been demonstrated to take place and these results are consistent with previous findings (Chapter 2). Although the Zn applied in 1983 had a sigmodial relationship to yield, the Mitscherlich equation adequately described all the yield responses to freshly and previously applied Zn (Table 6.1). Therefore, the Mitscherlich equation was used throughout to calculate \( b_c \), \( \text{RE}_{Zn} \) and \( \text{RR}_{\text{species}} \) for Zn applied in different years.

Relative to current Zn, there was a steady decline in the effectiveness of Zn as the length of time since the Zn was applied increased (\( \text{RE}_{Zn} \) values in Table 6.1). For example, relative to the Zn applied in the current year, Zn applied 14 years previously was about 34 % as effective for \( L. \text{angustifolius} \), about 35 % as effective for \( L. \text{luteus cv. Motiv} \) and only 4 % as effective for \( L. \text{luteus cv. Teo} \) (see the \( RE_{Zn} \) values in Table 6.1).

The decline in the RV of the three lupin species (measured in 1997) is compared with the decline in RV for wheat measured in this experiment in 1996 (Chapter 4.2) in Figure 6.2.
Figure 6.1 The relationship between grain yield and the amount of Zn applied for three lupin varieties: (a) *L. angustifolius* cv. Gungurru, and (b) *L. luteus* cv. Teo; and (c) *L. luteus* cv. Motiv. grown in 1997 when Zn was applied in 1983 (◆), 1986 (▲), and 1997 (■).
In Figure 6.2, the decrease in RV values for each of the 4 crops was fitted to a linear equation, and the slope values of the equations which quantify the rate of decrease in RV for grain production of each crops per year, were:

Wheat (1996) \(-0.038\).

*L. angustifolius* cv. Gungurru (1997) \(-0.048\).

*L. luteus* cv. Motiv (1997) \(-0.046\).

*L. luteus* cv. Teo (1997) \(-0.069\).

The rate of decrease in RV per year was least for wheat, followed by *L. angustifolius* and *L. luteus* cv. Motiv, which had a similar rate of decline in RV, and was largest for *L. luteus* cv. Teo. However, RV for wheat was determined in a different year to the lupins, and seasonal conditions may greatly influence RV values for Zn, as has been found when RV for P was determined in different years of the same experiment using grain yield of wheat at Wongan Hills, WA (Bolland 1999).

**Zinc oxide used by lupins (RRspecies values):** Relative to *L. angustifolius* cv. Gungurru (RE\(_{Zn}\) = 1.0), and for Zn applied in the current year (1997), *L. luteus* cv. Motiv was about half as effective in acquiring Zn for producing grain (RE\(_{Zn}\) = 0.56, see Table 6.1).

Therefore, about twice as much Zn was needed by cv. Motiv in the current year for it to produce the same percentage of maximum relative yield as cv. Gungurru. By contrast, relative to *L. angustifolius* cv. Gungurru, *L. luteus* cv. Teo was about 70 % more effective (RE\(_{Zn}\) = 1.70) and so, for current Zn, about 30 % less Zn needed to be applied for cv. Teo to produce the same relative yield as cv. Gungurru.
Figure 6.2 Decline in effectiveness of Zn applied in each of the previous years relative to the effectiveness of Zn applied in the current year (residual value or RV$_{Zn}$) for wheat and three lupins; *L. angustifolius* cv. Gungurru, *L. luteus* cv. Motiv, and *L. luteus* cv. Teo.

Relative to cv. Gungurru, *L. luteus* cv. Motiv used the previous Zn more effectively to produce grain, that is RR$_{\text{species}}$ values were always $>1.00$, ranging from 1.03 to 1.33 (see Table 6.1). However, *L. luteus* cv. Teo was less effective with RR$_{\text{species}}$ values always $<1.0$, decreasing from 0.85 for Zn applied in 1992 to 0.12 for Zn applied in 1983 (see Table 6.1).
Table 6.1. Values of the coefficients of the Mitscherlich equation \((a, b, c)\) fitted to the relationship between grain yield (kg/ha) and the amount of Zn fertiliser applied to derive estimates of the residual value \((RV_{Zn})\) and relative response \((RR_{species})\) values.

<table>
<thead>
<tr>
<th>Lupinus species</th>
<th>Yrs&lt;sup&gt;a&lt;/sup&gt;</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>(R^2)</th>
<th>bc</th>
<th>RV&lt;sub&gt;Zn&lt;/sub&gt;&lt;sup&gt;b&lt;/sup&gt;</th>
<th>RR&lt;sub&gt;species&lt;/sub&gt;&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. angustifolius&lt;sup&gt;d&lt;/sup&gt;</td>
<td>14</td>
<td>846</td>
<td>695</td>
<td>0.611</td>
<td>0.952</td>
<td>424.71</td>
<td>0.34</td>
<td>1.00</td>
</tr>
<tr>
<td>L. luteus cv Motiv</td>
<td>14</td>
<td>1007</td>
<td>308</td>
<td>0.795</td>
<td>0.863</td>
<td>244.75</td>
<td>0.35</td>
<td>1.03</td>
</tr>
<tr>
<td>L. luteus cv Teo</td>
<td>13</td>
<td>984</td>
<td>277</td>
<td>0.317</td>
<td>0.957</td>
<td>87.81</td>
<td>0.04</td>
<td>0.12</td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>13</td>
<td>839</td>
<td>677</td>
<td>0.646</td>
<td>0.972</td>
<td>437.34</td>
<td>0.35</td>
<td>1.00</td>
</tr>
<tr>
<td>L. luteus cv Motiv</td>
<td>13</td>
<td>1030</td>
<td>318</td>
<td>1.008</td>
<td>0.922</td>
<td>320.54</td>
<td>0.46</td>
<td>1.31</td>
</tr>
<tr>
<td>L. luteus cv Teo</td>
<td>13</td>
<td>987</td>
<td>265</td>
<td>0.479</td>
<td>0.956</td>
<td>126.94</td>
<td>0.06</td>
<td>0.17</td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>11</td>
<td>822</td>
<td>664</td>
<td>0.908</td>
<td>0.960</td>
<td>602.91</td>
<td>0.48</td>
<td>1.00</td>
</tr>
<tr>
<td>L. luteus cv Motiv</td>
<td>11</td>
<td>993</td>
<td>295</td>
<td>1.531</td>
<td>0.978</td>
<td>451.70</td>
<td>0.64</td>
<td>1.33</td>
</tr>
<tr>
<td>L. luteus cv Teo</td>
<td>11</td>
<td>960</td>
<td>260</td>
<td>1.096</td>
<td>0.953</td>
<td>285.02</td>
<td>0.13</td>
<td>0.28</td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>7</td>
<td>818</td>
<td>661</td>
<td>1.379</td>
<td>0.949</td>
<td>911.32</td>
<td>0.73</td>
<td>1.00</td>
</tr>
<tr>
<td>L. luteus cv Motiv</td>
<td>7</td>
<td>1008</td>
<td>290</td>
<td>1.954</td>
<td>0.941</td>
<td>566.75</td>
<td>0.81</td>
<td>1.11</td>
</tr>
<tr>
<td>L. luteus cv Teo</td>
<td>7</td>
<td>966</td>
<td>307</td>
<td>1.878</td>
<td>0.944</td>
<td>576.39</td>
<td>0.27</td>
<td>0.37</td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>5</td>
<td>810</td>
<td>609</td>
<td>1.500</td>
<td>0.986</td>
<td>913.38</td>
<td>0.73</td>
<td>1.00</td>
</tr>
<tr>
<td>L. luteus cv Motiv</td>
<td>5</td>
<td>1005</td>
<td>303</td>
<td>2.083</td>
<td>0.898</td>
<td>631.25</td>
<td>0.90</td>
<td>1.23</td>
</tr>
<tr>
<td>L. luteus cv Teo</td>
<td>5</td>
<td>986</td>
<td>279</td>
<td>4.754</td>
<td>0.997</td>
<td>1326.32</td>
<td>0.62</td>
<td>0.85</td>
</tr>
<tr>
<td>L. angustifolius</td>
<td>0</td>
<td>808</td>
<td>603</td>
<td>2.072</td>
<td>0.985</td>
<td>1249.24</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>L. luteus cv Motiv</td>
<td>0</td>
<td>1060</td>
<td>349</td>
<td>2.013</td>
<td>0.908</td>
<td>702.54</td>
<td>1.00</td>
<td>0.56</td>
</tr>
<tr>
<td>L. luteus cv Teo</td>
<td>0</td>
<td>1037</td>
<td>337</td>
<td>6.313</td>
<td>0.996</td>
<td>2127.48</td>
<td>1.00</td>
<td>1.70</td>
</tr>
</tbody>
</table>

<sup>a</sup> Years since Zn applied, 1997=0; 1992=5 years; 1990=7 years; 1986=11 years; 1984=13 years; 1983=14 years.

<sup>b</sup> For each lupin species, the effectiveness of Zn applied in the current and each of the previous years was calculated relative to the effectiveness of Zn applied in the current year (relative effectiveness or RE) to provide the \(RE_{Zn}\) values.

<sup>c</sup> The relative response (RR) of the different lupin species to Zn fertiliser was compared by dividing the bc of each of the species by the bc for L. angustifolius for current Zn to provide \(RR_{species}\) values. Therefore the \(RE_{species}\) for L. angustifolius grown with current Zn is, by definition, 1.00.

<sup>d</sup> L. angustifolius cv. Gungurru.

**Zinc concentrations in the grain:** Zinc concentrations in the grain from the nil-Zn treatment were (mg/kg): L. angustifolius 12; L. luteus cv. Motiv 15, cv. Teo 14, but increased with Zn additions. For example, in L. angustifolius, Zn values increased from 12 mg/kg for the nil Zn to 28 mg/kg for the highest amount of Zn applied. For L. luteus cv Teo, grain Zn increased from 14 mg for the nil Zn to 40 mg/kg for the highest amount of Zn applied. Zn concentration in grain of L. luteus was always about 40 % higher than in L. angustifolius grain.

There was a good relationship between the grain concentration of Zn and grain yield (data only shown for 1997) measured for all lupin species (Figure 6.3). The critical
concentrations of Zn in the grain were similar for the three lupins, being (mg/kg): *L. angustifolius* cv. Gungurru, 21; *L. luteus* cv. Motiv, 22; and *L. luteus* cv. Teo, 22.

![Figure 6.3](image.png)

**Figure 6.3** The relationship between the percentage maximum grain yield and Zn concentrations in the grain of *L. angustifolius* cv. Gungurru (■, solid line), *L. luteus* cv. Motiv (●, short dash), and *L. luteus* cv. Teo (▲, long dash) for 1997.

**Grain P and Cd concentration and the relationship with Cd and Zn in seed:** The concentration of P measured in the grain was unaffected by the amount of Zn applied in either the current year or in each of the previous years (Table 6.2). Compared with *L. angustifolius*, concentration of P was about two times higher in *L. luteus* seed and the concentration of Cd was five to seven times higher. For *L. angustifolius*, there was no relationship between the concentration of Cd in the seed and the concentration of Zn in the seed. However, for the two cultivars of *L. luteus*, the concentration of Cd in the grain decreased as the concentration of Zn in the grain increased. This suggests that as more fertiliser Zn is available from the current or previous years, less Cd accumulates in the
grain. For cv. Teo, the fitted equation was: \( y \ [\text{Cd mg/kg}] = 0.191 - 0.002 \ Zn \ [\text{mg/kg}] \), \( R^2 = 0.62 \) (P=0.001). The fitted model for the Motiv cultivar was: \( y \ [\text{Cd mg/kg}] = 0.295 - 0.005 \ Zn \ [\text{mg/kg}] \), \( R^2 = 0.58 \) (P=0.001). Note that, the concentration of Cd was measured in grain grown in 1997, so the Cd was derived from indigenous Cd in the soil and Cd added in fertiliser applied in previous years and the current year. Therefore, we cannot comment on the effects of P sources on Cd concentration in grain.

**Table 6.2.** Grain P concentration, expressed on dry basis, and Cd and Zn measured in grain for *L. angustifolius* cv. Gungurru, *L. luteus* cvv. Motiv and Teo. (Data are means for Zn applied in 1983 (se) \( n=18 \)).

<table>
<thead>
<tr>
<th>Lupin(^a)</th>
<th>Phosphorus</th>
<th>Zinc</th>
<th>Cadmium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>mg/kg</td>
<td>mg/kg</td>
</tr>
<tr>
<td>Gungurru</td>
<td>0.33 (0.02)</td>
<td>18.8 (1.0)</td>
<td>0.03 (0.002)</td>
</tr>
<tr>
<td>Motiv</td>
<td>0.58 (0.03)</td>
<td>26.5 (1.3)</td>
<td>0.21 (0.01)</td>
</tr>
<tr>
<td>Teo</td>
<td>0.57 (0.03)</td>
<td>26.5 (1.2)</td>
<td>0.18 (0.01)</td>
</tr>
</tbody>
</table>

\(^a\) *L. angustifolius* cv. Gungurru; *L. luteus* cvv Motiv and Teo.

**6.1.5 Discussion**

The level of Zn contamination in the superphosphate applied annually for 14 years supplied adequate Zn for the three lupins used in this study so that relative to current Zn applied as ZnO in 1997, it had a good residual value. This result is consistent with results of previous research for cereal crops (Chapter 4.1; Takkar and Walker 1993). Although the applications of Zn to the soil are low (0.6 to 1.0 kg Zn/kg), the use of superphosphate at >150 kg/ha has supplied sufficient amounts of Zn (90 g Zn/ha-per year) in soil to meet the requirements of the current crop (Chapter 4.1). Therefore, any decline in the effectiveness of the original ZnO application has been masked by the annual additions of Zn present as a contaminant in superphosphate, supporting results of Chapter 4.1 and Chapter 4.2. The RV of Zn applied as ZnO was quantified using yield of wheat in 1996 (Chapter 4.2) and yields of three lupins in 1997 (this Chapter). For ZnO, and the Zn present as a contaminant in superphosphate, the RV of Zn fertiliser is due to undissolved
Zn still present in the fertiliser, the Zn that has dissolved from the fertiliser and which is either retained by the soil or the Zn taken up by plants which is returned to the soil as organic matter as previously discussed (Chapter 4.2). The amount of Zn removed in product is typically low relative to the amount of Zn applied. In the present study, where 6 kg Zn/ha had been applied to the soil in 1983, it is estimated that about 5 % of the total Zn was removed from the soil as a result of grain being removed by the successive crops. The pasture was not grazed or defoliated so no Zn was removed from the soil-Zn system during those phases of the rotation. Losses of Zn from the soil system through leaching (Chapter 4.1) and erosion are negligible. Therefore, most of the residual Zn is present in the soil.

Zinc concentration in grain can be used as a post-mortem method of determining Zn deficiency of lupins; concentrations of 21 mg Zn/kg in lupin grain may suggest future applications of Zn fertiliser are required. Riley et al. (1992) found no further grain yield increase of L. angustifolius resulting from applications of Zn fertiliser where grain Zn concentration was 19 mg/kg. This study found that the critical level of Zn for L. angustifolius and L. luteus was similar, being 21 to 22 mg Zn/kg which is in agreement with Riley et al. (1992) for L. angustifolius; Riley et al. (1992) did not include L. luteus in their study.

The RV for L. luteus cv. Teo decreased more rapidly than the other two lupins. Different crop species, including L. angustifolius and L. luteus, have been shown to have different rhizosphere modification capacity with some species having more acidic rhizosphere pH values (Pearse et al. 2003). It could be that different cultivars of the same species have different pH values in their rhizosphere. Lower pH values in the rhizosphere may enhance mobilisation of Zn present in soil of the rhizosphere and so increase Zn uptake from soil.
In addition, different crop species, including *L. angustifolius* and *L. luteus*, have been shown to excrete different types and amount of carboxylates from roots into the root rhizosphere (Pearse *et al.* 2003). Similarly, different desi chickpea (*Cicer arietinum* L.) cultivars have been shown to secrete different amounts and types of carboxylates from roots into the root rhizosphere (Veneklaas *et al.* 2003). Carboxylates secreted from roots of *L. albus* into root rhizospheres have been shown to enhance the uptake of P from soil, particularly when the soil is low in P (Gardner *et al.* 1981, 1982a, b, 1983; Dinkelaker *et al.* 1989, 1995; Gerke *et al.* 1994; Gerke 1994; Hocking *et al.* 1997). The carboxylates may also enhance Zn uptake from soil. If cultivars of the same species differ in the amount and type of carboxylates exuded from plant roots into the root rhizosphere, then this may influence the effectiveness of residual Zn for the different cultivars of lupin in the present study. Romer *et al.* (2000) found that the roots of both *L. albus* and *L. angustifolius* secreted citrate. It is proposed that the citrate may desorb soil Zn, thereby increasing Zn uptake by both lupin species.

Different rhizosphere pH values, and exudation of different amounts and types of carboxylates from roots to the rhizosphere may both, in part at least, explain why *L. luteus* cv. Teo had a different rate of decline of RV for zinc than *L. luteus* cv. Motiv. Lupins differ from most agricultural plants in that they do not form mycorrhizal associations (Trinick 1977; Robson 1986; Gumley 1989). However, these and other mechanisms of lupin roots allowed them to adapt to poor nutrient status soils. For example, Bolland *et al.* (2000a) found that *L. luteus* used applied fertiliser P much more efficiently than *L. angustifolius*. Bolland *et al.* (2000a) found abundant third order laterals on secondary roots of *L. luteus*, but no such roots were found on *L. angustifolius*, leading the authors to suggest the third order lateral roots may have enhanced P uptake from soil by *L. luteus*. Gerke *et al.* (1994) reported similar third order lateral roots on *L. luteus* which they claimed were cluster roots, and demonstrated that the cluster roots on secondary *L. luteus*
roots secreted carboxylates. The development of cluster roots by *L. luteus* may increase the dissolution of insoluble soil Zn that may be taken up by cluster roots before it is retained by the soil. By contrast, *L. angustifolius* does not produce cluster roots (Clements *et al.* 1993). Both the *L. luteus* cultivars had higher Cd concentrations in grain than the *L. angustifolius* cultivar. Carboxylates secreted from third order lateral or cluster roots on secondary *L. luteus* roots may enhance uptake of Cd from soil. Bolland *et al.* (2000a) found that *L. luteus* cv. Teo had higher concentrations of potassium, sulfur, magnesium, copper and Zn in dried shoots and grain than *L. angustifolius* cv. Merrit. This was attributed to the abundant third order lateral roots (=cluster roots) found on secondary roots of *L. luteus*. Clements *et al.* (1993) found that the total length of roots for *L. luteus* was about 25 % longer than *L. angustifolius*. The root system of *L. angustifolius* consists of a dominant taproot with many primary laterals but few secondary and tertiary laterals (Clements *et al.* 1993). The number of primary lateral roots decreased rapidly with depth for *L. angustifolius*, but the distribution was more even for *L. luteus*. The total root length and the surface area of the roots have a profound effect on the uptake of nutrients, particular nutrients such as Zn that are immobile in the soil. These root mechanism seem to favour *L. luteus* compared to *L. angustifolius*, hence possible causes for the greater uptake of soil applied Zn. Once Zn is taken up from the soil by the plants, translocation of Zn to grain may be greater in *L. luteus* than in *L. angustifolius*. However, considering the mechanisms of Zn acquisition outlined above, the decline in the RV of Zn for *L. luteus* was unexpected. The root uptake, transport and utilisation of Zn within the *L. luteus* lupin plant require further glasshouse studies.

The lupin crop in the present Chapter showed larger yield responses to applied Zn and the decrease in RV was larger for the lupin than the wheat crop grown the previous year on the same plots (Chapter 4.2). There are three possible reasons for this result. First, the Zn removed in wheat grain would have reduced the amount of available Zn in soil for the
following lupin crop. However, the amount of Zn removed in the wheat grain is relatively small and unlikely to markedly reduce the RV of the Zn for the following lupin crop. Secondly, the Zn had one more year to react with soil and so become less available for the lupin crop. However, again one year is unlikely to have a marked effect on the RV of the Zn in soil. The combination of the first 2 possible causes may have induced a Zn response in lupin. If the Zn availability to the previous wheat crop (Chapter 5.1) was just adequate (and several wheat crops contained marginal leaf and grain Zn concentrations), the removal of Zn by wheat grain together with increase retention of Zn by soil could have been sufficient to drop available Zn levels to below the critical levels for the next lupin crop. The third possibility is the effect of seasonal conditions on plant yield responses to the Zn residues in soil. In a field study with phosphorus (P), Bolland (1999) compared the RV of P applied one year previously with the effectiveness of P applied in the current year in each of several growing season (years). The P applied as granulated single superphosphate was in the top 10 cm of soil which rapidly dried out between rains in the growing season. In the wet year, the one-year old P was in moist soil and was accessed by the wheat roots for longer. Consequently in the wet year the RV of the one-year old P was almost equal to that of the P applied in that year. In the driest year, the one-year old P was only one fifth as effective as the P applied that year. The one-year old P was in dry soil for longer and so not as much was accessed by roots relative to the current P in the dry year. This may also be the case for Zn applied in a previous year since Zn, like P, is immobile in the soil and remains in the surface layers where it is applied. In a wet year plant roots would be able to take up more Zn from soil than in a dry year, so RV of Zn in a dry year would be smaller than in a wet year. Further research is required to test the hypothesis that the RV of Zn would vary depending on growing season rainfall, as was found for P by Bolland (1999).

The comparison of *Lupinus* species and their Zn requirement is of importance for
agriculture in WA. However, a diverse range of grain-legumes are now being recommended for the neutral to alkaline soils of WA. The Zn requirements of these species are not known and experiments were done to compare the Zn requirements of the new legumes with wheat. The results of these experiments are presented in the next two sections of this Chapter.
6.2 Comparing grain legumes and wheat response to Zn

6.2.1 Abstract

Faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* M.) are being assessed as possible grain legumes for the neutral to alkaline soils of WA. The Zn requirements of these alternative grain legumes are not known. Therefore, the yield response of faba bean, chickpea, lentil, and wheat to applications of Zn fertiliser was compared in a glasshouse experiment using two alkaline soils from WA. Comparative Zn requirement was determined from yields of 46 day old dried shoots when no Zn fertiliser was applied, the amount of Zn required to produce the same percentage of the maximum (relative) yield, and the Zn content of dried whole shoots (Zn concentration multiplied by yield of dried shoots). The concentration of Zn in youngest mature growth (YMG) and in dried rest of shoots was used to determine critical concentrations for Zn in tissue. The external Zn requirement generally increased in the order faba bean < chickpea < wheat < lentil. Zinc concentration in youngest mature growth and in rest of shoots increased with an increase in the amount of added Zn. The critical Zn concentration in the YMG, associated with 90 % of the relative yield, was (mg Zn/kg): 25 for lentil, 18 for faba, 17 for chickpea and 12 for wheat; corresponding values for dried rest of shoots (mg Zn/kg) were: 30 for lentil, 20 for wheat, 19 for faba, and 17 for chickpea.

Faba bean (*Vicia faba* L.), chickpea (*Cicer arietinum* L.), and lentil (*Lens culinaris* M.) are being evaluated as possible grain legumes to grow in rotation with cereals on the fine textured neutral to alkaline soils in WA (Siddique *et al.* 1993). Zinc is a common deficiency of cereal crops grown in these soils (Gartrell and Glencross 1968; Brennan 1986, 1998, 2000). The Zn requirements of faba bean, chickpea and lentil grown in these soils are not known, and is the topic of the glasshouse experiment reported in this section. It appears that the Zn requirement of different crop species can vary greatly (Singh *et al.* 1983a; Graham and Rengel 1993). For example, chickpea appear to be more sensitive to Zn deficiency than cereals (Tiwari and Dwivedi 1990). The Zn requirements of wheat grown in all soil types in WA are well known (Gartrell and Glencross 1968; Brennan 2000). By relating the Zn requirements of new crop species to those for wheat, it is proposed to use the extensive amount of information available on the Zn requirements of wheat as a benchmark for assessing requirements of the new species.

6.2.3 Materials and Methods

**Soils:** The <4 mm fraction of the top 10 cm of two Zn deficient alkaline soils was used. Both soils are used to grow faba bean, chickpea and lentil in WA. The soils had never been fertilised and were collected from remnant vegetation areas where no previous fertiliser had been applied. The collection sites for the soils were near Salmon Gums [33°52’S, 121°40’E] and Mt Ney [33°40’S, 122°30’E] north and north east of Esperance [33°S, 121°54’E], about 650 km south east of Perth [32°S, 116°E]. Soil classification and some properties of the soils are listed in Table 6.3.

**Experiment:** The experiment comprised three replications of two soils (Kumarl and Ney soil, see Table 6.3), four plant species (faba bean, cv. Fiord; chickpea, cv. Tyson; lentil,
cv. Digger; wheat, cv. Brookton) and five amounts of Zn ((μg Zn/pot): 0 [Zn₀], 250 [Zn₁], 500 [Zn₂], 750 [Zn₃], and 1000 [Zn₄]).

Table 6.3. Soil classification, weight of soil used for each pot, and some properties of the top 10 cm of the <4 mm fraction.

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Salmon Gum</th>
<th>Mt Ney</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Classification:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Local name</td>
<td>Kumarl soil</td>
<td>Ney soil</td>
</tr>
<tr>
<td>Soil Survey Staff (1975)</td>
<td>Typic Eutrochrept</td>
<td>Typic Natrixeralf</td>
</tr>
<tr>
<td>Weight of soil per pot (g/pot)</td>
<td>2800</td>
<td>2650</td>
</tr>
<tr>
<td><strong>Soil properties:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8</td>
<td>8.7</td>
</tr>
<tr>
<td>Clay (%)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34</td>
<td>26</td>
</tr>
<tr>
<td>Organic carbon (%)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1.3</td>
<td>0.53</td>
</tr>
<tr>
<td>Iron oxide (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Aluminium oxide (%)&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Ammonium oxalate extractable Zn (mg/kg)&lt;sup&gt;e&lt;/sup&gt;</td>
<td>0.4</td>
<td>0.35</td>
</tr>
<tr>
<td>DTPA extractable Zn (mg/kg)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.3</td>
<td>0.1</td>
</tr>
<tr>
<td>Bicarbonate extractable P (mg/kg)&lt;sup&gt;g&lt;/sup&gt;</td>
<td>5</td>
<td>&lt; 4</td>
</tr>
<tr>
<td>CaCO₃ (%)&lt;sup&gt;h&lt;/sup&gt;</td>
<td>3</td>
<td>&lt; 2</td>
</tr>
</tbody>
</table>

<sup>a</sup> 1:5 soil:0.01M CaCl₂ (w/v), Rayment and Higginson (1992).
<sup>b</sup> Day (1965).
<sup>c</sup> Walkley and Black (1934).
<sup>d</sup> Sesquioxides (Hesse 1971).
<sup>e</sup> Gupta and McKay (1966).
<sup>f</sup> Lindsay and Norvell (1978).
<sup>g</sup> Colwell (1963).
<sup>h</sup> Rayment and Higginson (1992).

The pots were completely randomised in the glasshouse. About 2800 g of each soil (the amount of each soil used is listed in Table 6.3) was placed into plastic pots (17 cm diameter) lined with polyethylene bags. To ensure Zn was the only nutrient element limiting plant yield, the following solutions were added to the soil in each pot (mg/pot): NH₄NO₃, 250; K₂SO₄, 328; KH₂PO₄, 2200; MgSO₄.7H₂O 64; CaCl₂ 300, CuSO₄.5H₂O, 15; MnSO₄.5H₂O, 53; FeSO₄.7H₂O, 32, CoSO₄.7H₂O, 0.9; Na₂MoO₄.2H₂O, 0.8; and H₃BO₃, 1.0. On a surface-area basis, 227 mg/pot is equivalent to about 100 kg/ha. To remove any microelement contamination, including Zn, the macro nutrient salts were purified using dithizone-chloroform, as described in Chapter 2.1. Zinc was applied in
solution (5 mL/pot), as Zn sulfate (22.4 % Zn), at five amounts listed above. When the soils had dried after adding the solutions, the soil in each pot was thoroughly mixed by shaking the soil in the plastic bag.

The seed size was (mg/seed): spring wheat (36.3), faba bean (367), chickpea (130) and lentil (35). The Zn concentration in sown seed was (mg/kg): spring wheat (23), faba bean (24), chickpea (27) and lentil (28). The Zn content (seed size multiplied by Zn concentration) of the sown seed was (μg/seed): spring wheat (0.83), faba bean (8.8), chickpea (3.5) and lentil (1.0). Twelve seeds of each plant species were sown 1 cm deep in each pot before thinning to 5 plants per pot at 14 days after sowing. No inoculum was added so plants depended on added N fertiliser. For the first 14 days after sowing, soils were maintained at 75 % of field capacity using deionised water. Thereafter, the pots were maintained at field capacity by frequent watering to weight. The pots were re-randomised in the glasshouse after each watering. The glasshouse experiment was conducted between September and November. Temperatures in the glasshouse were set at 22°C day and 15°C night, ±1°C.

During the growth of the plants, nitrogen (purified ammonium nitrate, 227 mg/pot) was applied every 14 days.

**Measurements:** At 46 days after sowing, the plants were cut at ground level and the youngest growing tissue (youngest fully emerged leaf and the apical growth of each plant; YMG) was cut and separated from the remainder (rest) of the plants (Reuter *et al.* 1997a). The plant material was dried at 80°C for 48 hr and weighed. After weighing, the dried youngest tissue and the remainder of the whole shoots (ROS) were kept separate and were ground, digested and the concentration of Zn measured by atomic absorption spectrophotometry as outlined in Chapter 2.1.3.
**Analysis of data:** Data for the relationship between yield of dried whole shoots and the amount of Zn applied were described by a Mitscherlich equation:

\[ y = a - b \exp(-cx) \]  

where \( y \) is the yield (g/pot) of dried whole shoots (young growth plus the remainder of the dried shoots), \( x \) is the amount of Zn applied (µg Zn/pot) and \( a, b, c \) are coefficients. Coefficient \( a \) (g/pot) provides an estimate of the asymptote or maximum yield plateau. The value of the \( a \) coefficient was used as the maximum yield to calculate percentage of the maximum (relative) yield. Coefficient \( b \) (g/pot) estimates the difference between the asymptote and the intercept on the yield (y) axis at \( x = 0 \). Coefficient \( c \) (pot/g Zn) describes the shape of the relationship and governs the rate at which \( y \) (the yield response) increases as \( x \) (the amount of Zn applied) increases. Mean data were fitted to the equation by non-linear regression using a computer program written in compiler BASIC by procedures outlined in Chapter 6.1.3.

The different plant species produced different yields for the nil Zn treatment and different maximum yield plateaus so procedures outlined in analysis of data section of Chapter 6.1.3 were followed. The relative response (RR) of the different species to applications of Zn (RR\textsubscript{species}) was calculated by dividing \( bc \) of each species by \( bc \) of wheat. Therefore, by definition, the RR\textsubscript{species} of wheat is 1.00. Wheat was used as the standard species as it is widely grown on the same soils as the grain legume species studied here, and the Zn requirements of wheat for these soils are known, whereas the Zn requirement of the grain legumes is not.

Data for the relationship between Zn content of dried whole shoots (Zn concentration in dried whole shoots multiplied by the yield of the dried whole shoots) and the amount of Zn applied was adequately described by the linear equation:
where \( y \) is the Zn content in the dried whole shoots (µg Zn/pot), \( x \) is the amount of Zn applied (µg Zn/pot) and \( A \) and \( B \) are coefficients. Coefficient \( A \) provides an estimate of the Zn content of dried whole shoots derived from the soil when no Zn is applied (that is, the uptake of native soil Zn measured in the shoots). Coefficient \( B \) is the slope of the line, and estimates the increment of Zn content in dried whole shoots in the different plant species per unit of added Zn fertiliser.

The relationship between yield of dried whole shoots and the concentration of Zn in either dried youngest growth or in dried rest of shoots was used to define critical Zn concentrations in plants as outlined in Chapter 2. In this section, hand-fitted curves were used for the relationship between yield of dried whole shoots and the Zn concentration either in youngest mature growth or in dried rest of shoots to estimate critical Zn concentrations at 90 % of maximum shoot dry weight.

6.2.4 Results

_Symptoms of Zn deficiency_: Visual symptoms of Zn deficiency were observed three to five weeks after emergence of the plants. The symptoms of Zn deficiency were more evident for lentil and chickpea. Deficiency severely reduced lentil growth at three weeks after sowing and growth progressively worsened. The Zn deficient lentils were pale and stunted with leaf necrosis and premature shedding of leaves. At 5 weeks after sowing, the shedding of leaves was also particularly noticeable on chickpea at the nil-Zn and Zn\(_1\) level. Khan _et al._ (1998) also noted that Zn deficient chickpea shed leaves. At 5 weeks after sowing, faba bean on the Ney soil only were a paler green on the nil Zn treatment and the internode distances between the younger leaves was shorter. This ‘compressed’ younger growth resulting from Zn deficiency is often referred to as rosetting. The deficiency symptoms for wheat were a pale longitudinal strip along both sides of the mid-
rib of the youngest leaf. The deficient wheat plants were stunted and there was a reduction in growth after about 4 weeks; these symptoms were observed on the nil Zn and Zn₁ treatments, and are similar to symptoms observed in previous studies (Brennan 1986; Brennan et al. 1993).

*Dry matter production of whole shoots:* Except for faba bean on the Kumarl soil, yields of dried whole shoots of all plant species increased with increasing amounts of Zn applications. However, the magnitude of the yield increase varied with plant species and soil type (Fig. 6.3).

Dried whole shoots of faba bean showed no yield increase to added Zn on the Kumarl soil, so that all Zn treatments (Zn₀ to Zn₄) were on the maximum yield plateau (Fig. 6.4). For faba bean grown on the Ney soil, there was only about a 15 % increase in yield of dried whole shoots to the Zn₁ treatment, with the Zn₂ to Zn₄ treatments being on the maximum yield plateau.

Additions of Zn increased yields of dried chickpea whole shoots by about 30 % on the Kumarl soil and 20 % on the Ney soil. The increases only occurred up to the Zn₁ treatment, with the Zn₂ to Zn₄ treatments being on the maximum yield plateau.

Additions of Zn increased yields of dried whole shoots of wheat by about 40 % for the Kumarl soil and 35 % for the Ney soil. Increases in the dried weight of wheat shoots occurred up to the third level of Zn addition (Zn₄) with the plateau not clearly defined for both soils.

Increases in yield for dried whole shoots of lentil in response to Zn applications was about 45 % on the Kumarl soil and 65 % on the Ney soil, with increases in the dry weight of shoots occurring up to the largest amount of Zn applied (Zn₄) to both soils.
Comparing how the species use native and added Zn: The species differed in their ability to use native Zn in the soil to produce dried whole shoots (Fig. 6.4). Faba bean produced the largest absolute and relative yields of dried whole shoots for the nil Zn treatment (100 % for the Kumarl soil, 83 % for the Ney soil), followed by chickpea (70 % for the Kumarl soil and 80 % for the Ney soil), wheat (60 % for the Kumarl soil and 63 % for the Ney soil) and lentil (55 % for the Kumarl and 45 % for the Ney soil).
The yield response of dried whole shoots of the three legumes to the added Zn was compared to the yield response of wheat to the added Zn. The $RR_{\text{species}}$ values were smaller for lentil than wheat, being 0.40 for the Kumarl soil and 0.70 for the Ney soil (Table 6.4). This indicates that lentil used the Zn about 40 to 70% as effectively as wheat. Therefore, lentil required about 30 to 60% more Zn to produce the same percentage of the maximum (relative) yield as wheat.

**Table 6.4.** Values of the coefficients of the Mitscherlich equation\(^a\) fitted to the relationship between yield of dried whole shoots (g/pot) and the amount of Zn applied (µg Zn/pot), and, for each soil, relative response ($RR_{\text{species}}$).

<table>
<thead>
<tr>
<th>Species(^b)</th>
<th>$a$</th>
<th>$b$</th>
<th>$c$</th>
<th>$R^2$</th>
<th>$bc$</th>
<th>$RR_{\text{species}}$(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kumarl soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faba bean</td>
<td>nr(^d)</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
<td>nr</td>
</tr>
<tr>
<td>Chickpea</td>
<td>4.56</td>
<td>1.25</td>
<td>9.29</td>
<td>0.99</td>
<td>11.63</td>
<td>1.66</td>
</tr>
<tr>
<td>Lentil</td>
<td>2.61</td>
<td>1.49</td>
<td>1.89</td>
<td>0.99</td>
<td>2.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Wheat</td>
<td>8.77</td>
<td>3.6</td>
<td>1.95</td>
<td>0.98</td>
<td>7.02</td>
<td>1</td>
</tr>
<tr>
<td>Ney soil</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faba bean</td>
<td>11.8</td>
<td>1.77</td>
<td>9.45</td>
<td>0.98</td>
<td>16.7</td>
<td>2.55</td>
</tr>
<tr>
<td>Chickpea</td>
<td>6.13</td>
<td>1.25</td>
<td>7.91</td>
<td>0.99</td>
<td>9.92</td>
<td>1.52</td>
</tr>
<tr>
<td>Lentil</td>
<td>4.55</td>
<td>2.71</td>
<td>1.68</td>
<td>1</td>
<td>4.55</td>
<td>0.7</td>
</tr>
<tr>
<td>Wheat</td>
<td>9.14</td>
<td>3.63</td>
<td>1.8</td>
<td>1</td>
<td>6.54</td>
<td>1</td>
</tr>
</tbody>
</table>

\(^a\) Equation fitted: $y = a - b \exp (-cx)$, where $y$ is the yield of dried whole shoots (g/pot), $x$ is the amount of Zn applied (µg Zn/pot), $a$ provides an estimate of the maximum yield plateau (g/pot), $b$ is the yield response to added Zn (g/pot), and $c$ describes the shape of the relationship by estimating the rate at which $y$ approaches the maximum yield plateau as the amount of Zn applied is increased.


\(^c\) Calculated for each soil by dividing $bc$ (initial slope) for each species by $bc$ for wheat, so that, by definition, $RR_{\text{species}}$ for wheat is 1.00.

\(^d\) nr, no response, because faba bean on the Kumarl soil showed no yield response of dried whole shoots to applications of Zn fertiliser.

The $RR_{\text{species}}$ values of both faba bean and chickpea were larger than for wheat. This was so for chickpea on both soils and for faba bean on the Ney soil, indicating that the two legumes used the added Zn more effectively than wheat to produce dried whole shoots. Faba bean showed no yield response to Zn additions on the Kumarl soil so that the yields for all the Zn treatments were on the maximum yield plateau, presumably because faba bean obtained enough Zn from the indigenous Zn already present in the Kumarl soil to achieve maximum
yields. For both soils, the $R_{\text{species}}$ values for chickpea, 1.66 for the Kumarl and 1.5 for the Ney soil (Table 6.4), indicated that chickpea required about 40 to 50 per cent less Zn than wheat to produce the same relative yield of dried whole shoots. On the Ney soil, the $R_{\text{species}}$ value for faba bean was 2.55, indicating that faba bean required about one third of the amount of added Zn than wheat to produce the same relative yield of dried whole shoots.

Table 6.5. Values of the coefficients of the linear equation$^a$ fitted to the relationship between Zn content in dried whole shoots ($\mu g$ Zn/pot) and the amount of Zn applied ($\mu g$ Zn/pot). Numbers in brackets are standard errors ($n = 3$).

<table>
<thead>
<tr>
<th>Plant species$^b$</th>
<th>A</th>
<th>$B(10^2)$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kumarl soil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faba bean</td>
<td>194.8</td>
<td>26.37</td>
<td>0.984</td>
</tr>
<tr>
<td>Chickpea</td>
<td>50.9</td>
<td>7.02</td>
<td>0.944</td>
</tr>
<tr>
<td>Lentil</td>
<td>12.9</td>
<td>3.74</td>
<td>0.987</td>
</tr>
<tr>
<td>Wheat</td>
<td>55.4</td>
<td>13.05</td>
<td>0.987</td>
</tr>
<tr>
<td><strong>Ney soil</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faba bean</td>
<td>172.2</td>
<td>30.90</td>
<td>0.973</td>
</tr>
<tr>
<td>Chickpea</td>
<td>57.2</td>
<td>6.70</td>
<td>0.981</td>
</tr>
<tr>
<td>Lentil</td>
<td>13.3</td>
<td>6.78</td>
<td>0.983</td>
</tr>
<tr>
<td>Wheat</td>
<td>75.0</td>
<td>11.54</td>
<td>0.983</td>
</tr>
</tbody>
</table>

$^a$ Equation: $y = A + Bx$, where $y$ is the Zn content in dried whole shoots (Zn concentration in dried shoots multiplied by the yield of dried shoots) ($\mu g$ Zn/pot) and $x$ is the amount of Zn applied, $A$ is the intercept, which estimates the Zn content in dried shoots derived from indigenous Zn present in the soil when no Zn fertiliser is applied, and $B$ is the slope which estimates the increase in Zn content in dried shoots per unit of added Zn fertiliser. See Fig. 6.4 for the plotted mean values.

$^b$ Faba bean, Vicia faba cv. Fiord; Chickpea, Cicer arietinum cv. Tyson; lentil, Lens culinaris cv. Digger; Wheat, Triticum aestivum cv. Brookton.

Zn content of dried whole shoots: The relationship between Zn content in dried whole shoots (Zn concentration in dried whole shoots multiplied by the yield of the dried whole shoots) and the amount of Zn applied was linear for all four species and both soils (Fig. 6.5). For each amount of Zn added to both soils, faba bean had the highest Zn content in dried whole shoots, followed by wheat, then chickpea with lentil having the lowest Zn content of Zn in the dried whole shoots (Fig. 6.5). The same order was found for the value of the A and B coefficients of the linear equation fitted to the data (Table 6.5), indicating that faba bean achieved the largest Zn content from indigenous soil Zn and took up a greater proportion of the added Zn than the other species; lentil was the least
effective species at taking up indigenous and added Zn. Faba bean was equally effective on both soils in absorbing indigenous and applied Zn, whereas other species absorbed more on the Ney soil.

**Critical Zn concentration in tissue:** The relationship between yield of dried whole shoots (yield of young tissue + yield of rest of shoots) (dependent variable or y axis) and the Zn concentration in either dried youngest tissue or dried rest of shoots (independent variable or x axis) was different for the four species; this was so regardless of whether absolute or relative yield was used. The different species produced different maximum yield plateaus and different yields where no Zn was applied to both soils, so relative yield was used (Fig. 6.6). For wheat, faba bean and chickpea maximum yield (relative) plateaus were defined (Fig. 6.6). However, for lentil only one concentration was sufficiently high enough to achieved maximum yield. For faba bean only one concentration was less than the maximum yield plateau. Therefore, for lentil and faba bean the critical Zn concentration in young growth is not so accurately defined. However, the levels of Zn in young tissue represent a tentative concentration for diagnosis of Zn on farms of WA that will be refined with subsequent work in both glasshouse and field. The critical Zn concentration for diagnostic analysis in the young tissue was about (mg Zn/kg): 25 for lentil, 18 for faba bean, 17 for chickpea and 12 for wheat. Critical Zn values for dried rest of shoots (mg Zn/kg) were: 30 for lentil, 20 for wheat, 19 for faba bean and 17 for chickpea.
Figure 6.5 Relationship between the Zn content in dried whole shoots (µg Zn/pot) and the amount of Zn applied (µg Zn/pot) for (a) lentil, (b) faba bean, (c) wheat and (d) chickpea grown on the Kumarl (●) and Ney (■) soils. See Table 6.5 for fitted regression equations. Values plotted are means of three replicates. Please note the graphing program does not allow the use of µ, hence in Figure 6.4 the u = µ.

6.2.5 Discussion

The critical Zn values obtained in this study for young tissue and dried whole shoots are consistent with critical values obtained in previous studies. Critical values obtained for young tissues (mg Zn/kg) are: youngest fully emerged leaf blades of wheat, 10-12 (Brennan et al. 1993; Reuter et al. 1997a); leaves of faba bean (no indication of growth stage), 15-20 (Sandsted 1989); youngest faba bean leaves, 19 (Lewis and Hawthorne 1996). No critical Zn values for young tissue of lentil could be found in the literature. Critical values of Zn obtained for whole shoots in previous studies (mg/kg Zn) are: 45 day old lentil seedlings, > 32 (Tiwara and Dwivedi 1990); wheat, 20 (Radjagukguk et al. 1990).
The exception was for dried whole shoots of chickpea. Khan et al. (1998) obtained a critical value of 20-21 mg Zn/kg for dried whole shoots of 42 day old plants of 13 chickpea genotypes compared with a value of 17 mg/kg Zn for dried whole shoots of 46 day old cv. Tyson chickpea in this study.

**Figure 6.6** Relationship between percentage of the maximum (relative) yield of dried whole shoots and the concentration of Zn in dried young tissue (YMG) for faba bean, lentil, chickpea and wheat grown on Kumarl and Ney soils. *Note that data for both soils are on the same plot.*

The external Zn requirement of 46 day old plants generally increased in the order faba bean < chickpea < wheat < lentil. By contrast, the internal Zn requirement increased in
the order: wheat < chickpea < faba bean < lentil. Hence, the high external Zn requirement of lentil was in part explained by its high internal requirement. Pearse et al. (2003) reported low rates of organic anion (carboxylates) exudation (about 5 μmol/g root dry matter) for lentil grown under both low and high P supply. The exudates have been found to mobilise P in the soil and such mechanisms may increase Zn availability, but further research would be required to confirm this hypothesis. Chickpea and faba bean had similar internal Zn requirements for growth, but faba bean required much lower external Zn. Faba bean has a large root:shoot ratio (Pearse et al. 2003) which may explain its apparent efficacy in acquiring Zn from the soil. A large root: shoot ratio should enhance the uptake of most elements from the soil, especially those like Zn and P that are relatively immobile in the soil. However, P studies on faba bean suggest that they are not as P efficient as chickpea, albus lupin and wheat at using soil and fertiliser applied P (Bolland et al. 1999). Though faba bean has a large root: shoot ratio (Pearse et al. 2003), they have few fine roots (Mohammed Nuruzzaman and EJ Veneklaas, unpublished data). Despite having coarse roots, faba bean was very effective at accessing Zn from soil in this study. It could be that the Zn content in the relatively large faba bean seed supplied sufficient Zn during early growth in the glasshouse pot study reported here so there was negligible shoot yield responses to soil and applied Zn. However, three field experiments completed in WA also found negligible grain yield response of faba bean to applied fertiliser Zn (Bolland et al. 2000b). Further research is required to determine the Zn requirements of faba bean.

Chickpea, which had higher internal Zn requirements than wheat, required lower external Zn for maximum growth. This suggests that chickpea is relatively efficient in Zn uptake. Chickpea has high rates of organic anion (carboxylates) excretion into the rhizosphere along all the root surface, even on old roots, much higher than those in wheat (Pearse et al. 2003) and this trait may also enhance its capacity to acquire Zn (Marschner 1993).
Certainly higher P uptake efficiency in chickpea relative to wheat can be explained by higher organic anion levels in root exudates (Pearse et al. 2003; Veneklaas et al. 2003).

Therefore, this experiment indicated that the Zn requirement of chickpea and faba bean are likely to be lower than wheat based on seedling growth. The critical Zn concentration in both young tissue and whole shoots (= rest of shoots in this experiment) of these species grown on the neutral to alkaline soils of WA are methods by which possible Zn deficiencies of these species can be diagnosed. By contrast, lentil is expected to be substantially more sensitive to Zn deficiency than wheat. This has allowed the development of agronomic production packages in which Zn, a possible limiting nutrient for pulse production can be recommended for profitable grain production. However, this recommendation package for Zn fertiliser is based on seedling growth and requires verification in the field.

In field situations, the availability of soil Zn lower in the profile (sub-soil Zn) in relation to the rooting depth of each the grain legume species would need to be considered. This is not possible to determine in the present study, but further field research would be required to further enhance knowledge of Zn nutrition of the grain-legumes. Cereals are often affected by the root pruning effects of a group of herbicides, which induces Zn deficiency (Rudgers et al. 1970; Robson and Snowball 1989, 1990; Osborne and Robson 1992; Osborne et al. 1993). Herbicides within the Group D and Group B are used on grain legumes in WA (J Moore per. comm.). Both herbicide groups can prune roots and affect the uptake of Zn within the grain-legume crops of WA. Therefore, the root pruning effect on the Zn nutrition of the grain-legume species would be a significant consideration.

An understanding of the comparative Zn requirements of the newer crop species to be grown on an extensive range of soil types in WA is needed and the topic of the following section (Chapter 6.3).
6.3 Relative effectiveness of Zn for four crop species

6.3.1 Abstract

The newer crop species of canola (Brassica napus L.), albus lupin (Lupinus albus L.), and durum wheat (Triticum durum L.) are grown on a wide range of soil types of WA for grain production. The Zn requirements of these crop species are not known. Hence, the yield and Zn content response of canola, albus lupin, durum wheat and spring wheat (Triticum aestivum L.) to applications of Zn fertiliser was compared in a glasshouse experiment using two low Zn alkaline soils from WA. Five amounts of Zn applied as Zn sulfate were either added just before sowing (current Zn) or incubated in moist soil for 50 days (incubated Zn) before sowing seeds. The comparative Zn requirements were determined from yields of 40 day old dried shoots for: (i) Zn already present in the soil (indigenous Zn); (ii) the amount of fertiliser Zn required to produce the same percentage of the maximum (relative) yield of dried shoots; and (iii) the Zn content of dried shoots (Zn concentration multiplied by yield of dried shoots). The concentration of Zn in youngest tissue and in dried shoots was used to determine critical concentrations for Zn in tissue. Albus lupin used indigenous, current and incubated-Zn more effectively than canola, followed by spring wheat and then durum wheat. Albus lupin and canola were about 30 % and 40 % more effective at using fertiliser Zn than spring wheat. Durum wheat was about 20 % less effective than spring wheat. Relative to current Zn, the effectiveness of incubated Zn declined by about 60 % for both spring and durum wheat, and 50 % for canola and albus lupin.

Published as “Relative effectiveness of soil applied Zn for four crop species”. RF Brennan, MDA Bolland (2002), Aust. J. Exp. Agric. 42, 985-999.
The critical Zn concentration in the youngest mature growth (YMG), associated with 90% of the relative yield was (mg Zn/kg): 14 for spring wheat, 20 for durum wheat, 16 for albus lupin and 15 for canola. The corresponding values for critical Zn in dried rest of shoots (mg Zn/kg) was: 32 for spring wheat, 25 for durum wheat, 22 for albus lupin, and 23 for canola.

6.3.2 Introduction

Many of the neutral to alkaline soils, which comprise about 25% of the total 18 million hectares used for agriculture in WA, are low in Zn. Continuous cropping of spring wheat in rotation with field pea (*Pisum sativum* L.) is the most common rotation on the neutral to alkaline soils. The Zn requirements of wheat grown in all soil types in WA are well known (Gartrell and Glencross 1968; Brennan 2000). Recently, durum wheat (*Triticum durum* L.), canola (rapeseed, *Brassica napus* L.) and albus lupin (*Lupinus albus* L.) have shown promise as new crops for these soils.

Zinc applied to soil may react with inorganic and organic components of the soil. The effect of these reactions on the availability of Zn to plants is not well understood. Moist incubation of Cu with soil reduced its availability relative to freshly applied Cu (Brennan *et al.* 1980). In this study, the opportunity was taken to incubate Zn with the soil to examine possible effects on the availability of Zn for durum wheat, canola and albus lupin compared to spring wheat.

The Zn requirement of spring wheat grown in all soil types in WA has been determined. The Zn requirements of albus lupin, durum wheat and canola grown in these soils are not known. The Zn requirement of different crop species can vary greatly as described in Chapter 6.2.2. Therefore, the Zn requirements of new crop species are benchmarked against those for wheat, to expand the amount of information available on the Zn requirements of the new species. This section reports the result of a glasshouse pot study.
conducted to compare the Zn requirements of albus lupin, canola and durum wheat with that of spring wheat.

6.3.3 Materials and Methods

**Soils**: Two alkaline soil types known to be deficient in Zn for spring wheat production and also suitable for growing canola, albus lupin and durum wheat were used for the experiment. The soils were collected from sites as outlined in Chapter 6.2.3. Soils were collected from locations adjacent (within 50 m) to the soils used in Chapter 6.2 and soil properties varied to those previously measured in that chapter (compare Table 6.3). Soil classification and some properties of the soils are listed in Table 6.6.

<table>
<thead>
<tr>
<th>Soil classification and properties</th>
<th>Soil classification</th>
<th>Local name</th>
<th>Soil Survey Staff (1975)</th>
<th>Soil properties</th>
<th>Experimental design and growing conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soil classification</td>
<td>Salmon Gums</td>
<td>Ney</td>
<td>Typic Eutrochrept</td>
<td>Typic Natrixeralf</td>
<td>The experiment comprised 3 replications of 2 soils (Kumarl and Ney soil, see Table 6.6), 4 plant species (canola cv. Karoo; albus lupin cv. Kiev Mutant; spring wheat cv. Brookton, durum wheat cv Tamaroi) and 5 amounts of Zn as solutions of Zn sulfate ($\text{ZnSO}_4\cdot\text{7H}_2\text{O}$) ($\mu\text{g Zn/pot}$): 0 ($\text{Zn}_0$), 250($\text{Zn}_1$), 500 ($\text{Zn}_2$), 750 ($\text{Zn}_3$), and 1000 ($\text{Zn}_4$).</td>
</tr>
<tr>
<td>Local name</td>
<td>Kumarl soil</td>
<td>Ney soil</td>
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<tr>
<td>Soil Survey Staff (1975)</td>
<td>Typic Eutrochrept</td>
<td>Typic Natrixeralf</td>
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<tr>
<td>Soil properties</td>
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<td>pH&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td>Ammonium oxalate extractable Zn (mg/kg)&lt;sup&gt;e&lt;/sup&gt;</td>
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<td>0.32</td>
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<td></td>
</tr>
<tr>
<td>DTPA extractable Zn (mg/kg)&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.28</td>
<td>0.12</td>
<td></td>
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</tbody>
</table>

<sup>a</sup> 1:5 soil:0.01mol/L CaCl$_2$ (w/v) (Rayment and Higginson 1992).
<sup>b</sup> Day (1965).
<sup>c</sup> Walkley and Black (1934).
<sup>d</sup> Hesse (1971).
<sup>e</sup> Gupta and McKay (1966).
<sup>f</sup> Lindsay and Norvell (1978).
The pots were completely randomised in the glasshouse. The amount of soil used per pot was 2750 g for the Kumarl soil and 2650 g for the Ney soil. The soils were placed in 17 cm diameter by 19 cm depth plastic pots lined with polyethylene bags. Basal nutrient solution applications and macronutrient salt (N, K, P, Mg, and Ca) purification were as outlined in Chapter 6.2.3.

Zinc was added before or after incubation. All pots for all Zn treatments (including soils treated with Zn either before or after incubation) were watered to field capacity with deionised water and incubated at 22°C for 50 days. During incubation, each pot was sealed in polyethylene bags to prevent water loss from the moist soil in the pots. After incubation, the soil in the pots was air-dried before Zn solutions were added to those pots receiving Zn after the incubation. When the soils had dried after adding the current Zn treatments, the soils in each pot were again thoroughly mixed as outlined above. The Zn treatments, which were added after the incubation treatment, are referred to as the “current” Zn. The Zn treatments added before the incubation treatment are referred to as “incubated” Zn.

The seed size was (mg/seed): spring wheat (36.3), durum wheat (36.7), canola (3.0) and albus lupin (390). The Zn concentration in sown seed was (mg/kg): spring wheat (22), durum wheat (21), canola (20) and albus lupin (19). The Zn content (seed size multiplied by Zn concentration) of the sown seed was (μg/seed): spring wheat (0.79), durum wheat (0.76), canola (0.06) and albus lupin (7.6). Twelve seeds of each plant species were sown 1.5 cm deep in each pot before thinning to 5 plants per pot at 20 days after sowing. Procedures followed during the growth of the plants were as outlined in Chapter 6.2.3. The temperatures in the glasshouse were set at 21°C day and 15°C night, ±1°C for the August to September growing period.
During the growth of the plants, nitrogen (as purified ammonium nitrate at 250 mg/pot) was applied every 14 days.

**Measurements:** At 40 days after sowing, the plants were cut at ground level and the young growth (apex and the youngest emerged leaf) of each plant (YMG) was cut and separated from the remainder of the shoots (ROS). The total dried weight of shoots (DWS) was the addition of the dried weight of YMG and ROS. The plant material was dried, digested and the concentration of Zn was measured as described in Chapter 2.1.3.

**Analysis of data:** Data for the relationship between yield of dried shoots and the amount of Zn applied were adequately described by a Mitscherlich equation:

\[ y = a - b \exp(-cx) \]  \hspace{1cm} (1)

where \( y \) was the total yield (g/pot) of dried shoots (DWS, sum of yield of YMG and ROS), \( x \) was the amount of Zn applied (µg Zn/pot) and \( a, b, c \) are coefficients as described in the data statistical section of Chapter 6.2.3. The relative response (RR) of the different species to applications of Zn (RR\text{species}) was calculated as outlined in Chapter 6.2.3. In addition, the RR\text{species} was calculated separately for the current and incubated Zn treatments in this section. The RR for freshly applied Zn is defined as FRR\text{species}; the RR for incubated Zn is defined as IRR\text{species}.

For each species, the effectiveness of incubated Zn was calculated relative to the effectiveness of current Zn, by dividing the c coefficient for the incubated Zn by the c coefficient for current Zn, to provide relative effectiveness (RE) values for the incubated Zn.

The relationship between yield of DWS, expressed as relative yield, as the dependent variable (y axis), and the concentration of Zn in either YMG or in ROS, as the independent variable (x axis), was used to define critical Zn concentrations in tissue as
previously outlined in Chapter 2.1.3.

6.3.4 Results

**Symptoms of Zn deficiency:** Deficiency severely reduced spring wheat and durum wheat growth at 3 weeks after sowing and growth progressively worsened thereafter. Visual symptoms of Zn deficiency were observed about 4 weeks after emergence of the plants. The symptoms occurred earlier and were more severe for spring wheat and durum wheat than for albus lupin and canola. The deficiency symptoms for spring wheat were as described in Chapter 6.2.4. Symptoms of Zn deficiency in durum wheat were comparable to those symptoms seen in spring wheat (Chapter 6.2.4). Reduced internode length for albus lupin was observed on the Zn₀ treatment. Distortion and interveinal chlorosis of the youngest leaves were observed for the canola grown on the Zn₀ treatment.

**Dry matter production of shoots:** Yield of DWS of all plant species increased with increasing amounts of Zn applied. However, the magnitude of the yield response varied with plant species, soil type and for incubated and current Zn treatments (Fig. 6.6, Table 6.7). Per unit of applied Zn, compared to the current Zn treatments, incubation decreased yields of shoots, except for albus lupin grown on the Kumarl soil (Table 6.7). Therefore, for each species, the RE values for incubated Zn were <1.0, except for albus lupin on Kumarl soil.

The DWS of albus lupin showed a small yield response (<10 %, see (b/a)*100 values - Table 6.7) to added Zn on the Kumarl soil, so that the Zn₁ to Zn₄ treatments were all on the maximum yield plateau (Fig. 6.6). For albus lupin on the Ney soil, there was only about a 12 % increase in yield of DWS to the Zn₁ treatment, with the Zn₂ to Zn₄ treatments being on the maximum yield plateau.
Table 6.7. Values of the coefficients of the Mitscherlich equation\(^a\) fitted to the relationships between yield of dried shoots (DWS) (g/pot) and the amount of Zn applied (µg Zn/pot), and for either current (F) or incubated (I) Zn response of each species relative to Spring wheat (RR\(_{\text{species}}\)), and for each species, effectiveness (RE\(_{\text{Zn}}\)) of incubated Zn relative to current Zn. The coefficient of determination (R\(^2\)) was >0.97 in all cases.

<table>
<thead>
<tr>
<th>Species(^b)</th>
<th>Zn trt(^c)</th>
<th>DWS at nil Zn (x10(^3))</th>
<th>Relative Response (%)</th>
<th>bc</th>
<th>FRR(_{\text{species}}) (^d)</th>
<th>IRR(_{\text{species}}) (^e)</th>
<th>RE(_{\text{Zn}}) (^f)</th>
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<tr>
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<td>3.46</td>
<td>49.6</td>
<td>7.88</td>
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<tr>
<td></td>
<td>I</td>
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<td>4.69</td>
<td>2.46</td>
<td>1.38</td>
<td>52.5</td>
<td>3.39</td>
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<td><strong>Ney soil</strong></td>
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<td>2.44</td>
<td>1.65</td>
<td>54.7</td>
<td>4.03</td>
</tr>
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\(^{a}\)Equation fitted \(y = a - b \exp(-cx)\).
\(^{b}\)Canola, Brassica napus cv. Karoo; Albus lupin, Lupinus albus cv. Kiev mutant; Durum wheat, Triticum durum cv. Tameroi; Spring wheat, Triticum aestivum cv. Brookton.
\(^{c}\)Zinc added before the incubation (I); Zinc added after incubation (F).
\(^{d}\)Calculated for the current Zn treatments for each soil, by dividing bc (initial slope) of each species by bc for spring wheat, so that, by definition, FRR\(_{\text{species}}\) for spring wheat is 1.00.
\(^{e}\)Calculated for the incubated Zn treatments of each soil, by dividing bc of each species by bc for spring wheat, so that, by definition, IRR\(_{\text{species}}\) for spring wheat is 1.00.
\(^{f}\)Calculated for each species of each soil by dividing bc for the incubated Zn by bc of the current Zn, to provide effectiveness of incubated Zn relative to current Zn applied just before sowing seed.
\(^{g}\)Not determined (nd) as there was <9 % yield increase to Zn fertiliser.

Additions of Zn increased yields of DWS of canola by about 30 % on the Kumarl soil and 25 % on the Ney soil. For current Zn, the increases only occurred up to the Zn\(_1\) treatment, whereas the increase occurred up to the Zn\(_3\) treatment for the incubated Zn treatment. Increases in yield of DWS of durum wheat in response to Zn applications were about 40 % on the Kumarl soil and 60 % on the Ney soil, with increases occurring up to the largest amount of Zn applied (Zn\(_4\)) to both soils and for both current and incubated Zn.
Additions of Zn increased yields of DWS of spring wheat by about 50% for both the Kumarl and Ney soils. Yield of DWS of spring wheat increased to the third level of Zn.
addition (Zn₃) on both soils for both current and incubated Zn treatments.

*Indigenous Zn versus added Zn:* The species differed in their ability to use indigenous Zn in the soil to produce DWS (Fig. 6.6). Albus lupin produced the largest relative yields of DWS for the nil Zn treatment (about 92 % of the maximum for the Kumarl soil, and 76 % for the Ney soil), followed by canola (69 % for the Kumarl soil and 75 % for the Ney soil), spring wheat (50 % for both the Kumarl and Ney soils) and durum wheat (62 % for the Kumarl soil and 40 % for the Ney soil).

The yield response of DWS of the durum wheat, albus lupin and canola to current Zn was compared to the yield response of spring wheat to provide FRR<sub>species</sub> values. The FRR<sub>species</sub> value (Table 6.7) is, by definition, 1.00 for spring wheat. The FRR<sub>species</sub> values for durum wheat were about 0.73 for the Kumarl soil and 1.34 for the Ney soil (Table 6.7). Therefore, durum wheat used fresh Zn about 25 % as effectively on the Kumarl soil as, and about 34 % more effectively on the Ney soil than, spring wheat to produce the same relative DWS. This variation in the amounts of Zn required by durum wheat compared to spring wheat requires further clarification.

The FRR<sub>species</sub> values of both albus lupin and canola were larger than for spring wheat. This occurred for albus lupin on the Ney soil and for canola on both soils, indicating that both albus lupin and canola used current Zn more effectively than spring wheat to produce the same relative DWS yield. Albus lupin showed a small yield response, (<10 %; Table 6.7) to additions of current Zn on the Kumarl soil so that the yields were on the maximum yield plateau for current Zn₁ to Zn₄ treatments, presumably because albus lupin obtained enough Zn from the native Zn already present in the Kumarl soil. On the Ney soil, the FRR<sub>species</sub> value for albus lupin was 1.27, indicating that albus lupin required about three quarters the amount of current Zn than spring wheat to produce the same relative yield of DWS. For both soils, the FRR<sub>species</sub> values for canola, 1.74 for the Kumarl and 1.17 for
the Ney soil (Table 6.7), was about one and a half times larger (mean value for the soil types) than the $RR_{\text{species}}$ for spring wheat, indicating that canola required about 33 % less current Zn than spring wheat to produce the same relative yield of DWS.

The $IRR_{\text{species}}$ value for durum wheat was about 0.73 for the Kumarl soil and 1.14 for the Ney soil (Table 6.7). This indicates that durum wheat used the incubated Zn about 25 % as effectively on the Kumarl soil and 10 % more effectively on the Ney soil as spring wheat to produce the same relative DWS. The $IRR_{\text{species}}$ values of both albus lupin and canola were larger than for spring wheat, indicating that both albus lupin and canola used the incubated Zn more effectively than spring wheat to produce relative DWS yield. Albus lupin showed a small yield response, to additions of incubated Zn on the Kumarl soil so that for the incubated Zn$_1$ to Zn$_4$ treatments the yields were on the maximum yield plateau. On the Ney soil, albus lupin required about half the amount of incubated Zn than spring wheat to produce the same relative yield of DWS. For both soils, the $IRR_{\text{species}}$ values for canola, 2.22 for the Kumarl and 1.39 for the Ney soil (Table 6.7), indicated that canola required about 30-55 % less incubated Zn than spring wheat to produce the same relative yield of DWS.

Incubation decreased the effectiveness of applied Zn (see $RE_{\text{Zn}}$ of Table 6.7) measured by shoot yield for all 4 species grown on both soils. The magnitude of the decrease differed for the 4 plant species and 2 soils. For both soils, the decrease in RE value was smallest for albus lupin, and increased in the order albus lupin <canola <spring wheat = durum wheat for both soil types (Table 6.7).

Incubation decreased the effectiveness of applied Zn measured by Zn content for all 4 species grown on both soils. The magnitude of the incubation effect differed between plant species and soil. Albus lupin and canola had the largest RE values for incubated Zn measured using Zn content of DWS ($RE_{\text{ZN}}$ values higher see Table 6.7) while spring
wheat and durum wheat had significantly lower $RE_{Zn}$ values for both soils. The $RE_{Zn}$ values were largest for albus lupin and decreased in the order albus lupin > canola > spring wheat = durum wheat for both soil types. The $RE_{Zn}$ values for the Kumarl soil were higher than the $RE_{Zn}$ values measured for the Ney soil.

![Graphs showing relationship between relative yield and Zn concentration for spring wheat, durum wheat, canola, and albus lupin grown on two soils.](image)

**Figure 6.8** Relationship between percentage of the maximum (relative) yield of dried shoots of (a) spring wheat, (b) durum wheat, (c) canola and (d) albus lupin grown on 2 soils and the concentration of Zn in dried young tissue (new growth, YMG) and the Zn concentration in whole shoots. In each case solid line is for YMG; and dashed line is for whole shoots. Hand fitted curves.

**Critical Zn concentration in tissue:** The relationships between yield of DWS and Zn
concentration in either YMG or ROS were different for the 4 species; this occurred regardless of whether absolute or relative yield was used. The species produced different maximum and minimum yields on both soils and hence relative yield was used (Fig. 6.8). The Zn concentration in YMG that was associated with 90% of the maximum yield (critical Zn for diagnosis) was higher for durum wheat (about 20 mg Zn/kg) than for albus lupin (16 mg Zn/kg), canola (15 mg Zn/kg) or spring wheat (14 mg Zn/kg). Critical Zn value for ROS (mg/kg Zn) was: 25 for durum wheat, 32 for spring wheat, 22 for albus lupin and 23 for canola.

6.3.5 Discussion

The external Zn requirement of 42 day old plants was least for albus lupin and generally increased in the order albus lupin < canola < spring wheat = durum wheat. The Zn content was largest for sown albus lupin seed and that may explain the smaller yield increase to soil applied Zn. Rengel and Graham (1995c, d) found that the seed content rather than the concentration of Zn in the seed was more important for the following growth and yield of wheat grown on a Zn deficient soil. However, the sown canola seed had the smallest Zn content yet it showed a smaller yield increase to soil applied Zn than spring and durum wheat. All species contained similar Zn concentration in seeds, which seems to rule out a confounding of the response of species to Zn due to seed Zn level (Bell 2000).

The incubation of Zn with moist soil decreased the availability of Zn for all four plant species. The reduction in the availability of Zn is attributed to retention of Zn by soil constituents (organic matter, clay, iron and aluminium oxides) (see Chapter 2 & 3). The plant species varied in their ability to take up indigenous, current and incubated Zn. For each plant species, there was a common relationship between dried weight of shoots and Zn concentration in the youngest emerged tissue for current and incubated Zn treatments. This means that the youngest tissue is an effective means by which the Zn status of the
species can be determined. Other workers (see review Brennan et al. 1993) have illustrated that the youngest leaf tissue is an appropriate means to measure the status of the plant. If the relationship was different (2 separate well defined curves) for fresh and previously applied Zn, tissue testing for Zn by means of young leaves would be inappropriate and further work would be required to determine plant parts which best indicated Zn status.

In the literature, we could find no studies comparing, in the same experiment, the Zn response of canola, albus lupin, durum wheat and spring wheat to native soil Zn and Zn fertiliser added to the soil. This study showed that albus lupin used both sources of Zn more effectively than the other 3 species studied to produce dried shoots and increase Zn content in the shoots; durum wheat was the least effective. Both albus lupin and canola were more effective at using indigenous and fertiliser Zn than spring wheat or durum wheat. Albus lupin has been found to form cluster roots (Clements et al. 1993), have an extensive root system (measured as total root length) (Clements et al. 1993) and exude carboxylates (Pearse et al. 2003) which have been found to mobilise P and could be expected to help in the uptake of Zn (Marschner 1993). Durum wheat used both current and incubated Zn less effectively than spring wheat. Cakmak et al. (2001) illustrated that a range of durum wheat varied in their efficiency for Zn uptake. It was found that the difference in tolerance was related to the total amount of Zn per shoot rather than the concentration of Zn in the shoot. Durum wheat also possesses a lower capacity to take up Zn from soil and transport it into the shoots under low Zn supply (Erenoglu et al. 1999, 2002). The genotypic differences were inherent and not related to the seed content. Cakmak et al. (2001) suggested that relative shoot growth (Zn efficiency ratio) can be used to screen cultivars. However, Kalayci et al. (1999) suggested that measurement of Zn in plant parts of wheat cultivars, such as young tissue, might be a better indicator of genotypic differences. Wheat has low Zn efficiency; there are differences in Zn
efficiency between cultivars (Cakmak et al. 1996c; Graham et al. 1992; Kalayci et al. 1999; Rengel and Graham 1995a; Shukla and Raj 1974; Torun et al. 2001). Variations in Zn efficiency among wheat cultivars have been attributed to differences in uptake, possibly due to release of organic compounds from the roots or differences in root surface area (Cakmak et al. 1994; Rengel and Wheal 1997).

The critical tissue test values obtained in this study were similar to values obtained for each species studied separately in various previous studies. Critical values obtained for young tissue in previous studies (mg/kg Zn) were: youngest fully emerged leaf blades of spring wheat, 14 (Wilhelm et al. 1993; Brennan et al. 2001; Reuter et al. 1997a); leaves of albus lupin (no growth stage provided), 15-20 (Weir and Cresswell 1994); youngest open canola leaves, 15-17 (Huang et al. 1995). Critical values obtained for DWS (mg/kg Zn) were for canola seedlings, >32 (Rashid et al. 1994); spring wheat, 20 (Radjagukguk et al. 1980; Brennan et al. 1993; Reuter et al. 1997a; Brennan et al. 2001). No critical Zn values for youngest fully emerged leaf blades, ROS or whole shoots of durum wheat plants are reported in the literature.

6.3.6 Conclusion

Results demonstrate that four crop species tested have differential ability to use indigenous and soil applied Zn. External Zn requirement of 42 day old plants were least for albus lupin and generally increased in the order albus lupin <canola <spring wheat = durum wheat. Critical Zn concentration in the youngest growth was (mg Zn/kg) 20 for durum wheat, 16 for albus lupin, 15 for canola and 14 for spring wheat. The critical Zn concentration in dried shoots (mg Zn/kg) was 25 for durum wheat, 18 for albus lupin, 22 for canola and 32 for spring wheat.

The research work in this thesis clearly demonstrates that adequate Zn supply is essential to the economical production of a range of crop species in WA on typical neutral-alkaline
loamy soils. Spring wheat does appear to be a good indicator plant for the agricultural production system of WA. Cakmak et al. (1996a, 1998) categorised wheat as a cereal of low Zn efficiency. Although wheat has low Zn efficiency, there are significant differences in Zn efficiency among cultivars (Cakmak et al. 1996c; Graham et al. 1992; Kalayci et al. 1999; Rengel and Graham 1995a; Shukla and Raj 1974; Torun et al. 2001). However, in this study cultivar effects were not examined. Although Zn fertiliser once applied to the soil at recommended levels for the soil types appears to have a long residual value, the implications of cultivar efficiency for Zn fertiliser use in WA would need to be considered. The Zn efficient cultivars can also contribute to overcoming Zn deficiency problems related to inadequate subsoil supply that cannot be solved with usual fertiliser application practices (Graham et al. 1992; Nable and Webb 1993). In most cases the soil surface Zn (0-10 cm) can not efficiently meet the crop’s requirements because most topsoils in WA are sandy and frequently dry out between rains during the growing season, and Zn is very immobile in soil and so does not move down the soil profile (Grewal et al. 1997a; Grewal and Graham 1999). These findings need to be confirmed in field studies and extended to deal with a range of efficient and inefficient cultivars in WA soils. A general discussion of the results of this thesis is provided in the following chapter.
Chapter 7

General Discussion and Conclusions

7.1 Factors affecting the residual value of Zn in soils

The aims of this thesis were to study the various soil properties that affect the availability of Zn to plants. In this chapter the various factors that affect the availability and hence the residual value of Zn are discussed. A brief overview of the findings of this thesis is presented and also an overview of the residual value of Zn in agricultural systems of WA is discussed.

In glasshouse studies, incubation of Zn with warm moist soil reduced uptake of Zn by plants and, relative to freshly applied Zn (residual value of previously-applied Zn), decreased the effectiveness of Zn for production of dried shoots for a range of plant species. In field experiments, relative to freshly-applied Zn, the effectiveness of Zn applied in a previous year (residual value of fertiliser Zn applied to soil) decreased the longer the Zn was in soil due to continued reaction of the Zn with soil. The reduction in the residual value (RV) of Zn in both glasshouse and field studies varied with soil type and is attributed to precipitation of insoluble Zn from soil solution, adsorption of Zn by exposed iron and aluminium ions at the surfaces of soil constituents, and chelation of Zn by soil organic matter (Barrow 1993). These reactions of Zn in soil reduced Zn uptake by plant roots and hence the effectiveness of the Zn for plant production.

The soils used for the RV studies had diverse soil properties. The rate of decline in the RV of Zn was related by simple and stepwise multiple linear regression to soil properties. Simple linear regression indicated that the decline in RV of Zn was reasonably well predicted by three soil properties: soil pH, % clay and % organic carbon. In some alkaline soils the % free calcium carbonate also helped predict the decline in RV of Zn.
Stepwise multiple linear regression indicated that soil pH$_{Ca}$, % clay and % organic carbon together accounted for about 80% of the variation in RV of fertiliser Zn in the WA soils tested.

Other studies have also identified soil pH$_{Ca}$, clay content (%), organic carbon content (%) and free calcium carbonate (%) as factors influencing Zn availability or plant yield responses to applied Zn for different crop species (Martens et al. 1966; Martens 1968). For example, Martens (1968) found that an increase in soil pH, organic matter and clay content decreased the uptake of Zn by corn plants. However, the approach taken in this study was to relate the decline in RV of Zn to the measured soil properties for a range of soils, an approach not previously used in published research work. Therefore, it is highly likely that the RV of Zn would be low in soils of high soil pH, with high organic matter and clay content and with free calcium carbonate present in the soil. Few soils in WA have free calcium carbonate (McArthur 1991) so soil pH$_{Ca}$, and clay and organic carbon content of soil are the major soil properties explaining most of the variation in the RV of Zn in WA soils.

Previous research with cereal crops in WA has shown that regular (usually annual) application of >150 kg superphosphate/ha when contaminated with 400-600 mg Zn/kg supplied sufficient amounts of Zn to meet the requirements of the current crop. These additions of superphosphate have evidently maintained adequate Zn levels in WA soils despite any decline in the effectiveness of the original Zn application as ZnO fertiliser (Chapter 4; Brennan 1998, 2000). The residual effectiveness of Zn fertiliser in the soil is due to: (i) insoluble Zn compounds that remain in fertiliser particles previously applied to soil (Gilkes and Sadlier 1981), (ii) Zn dissolved from the fertiliser which is either retained by the soil or Zn taken up by plants and organisms growing in the soil and incorporated in the soil organic pools. Losses in residual effectiveness are due to continuing soil Zn reactions (discussed above) and Zn removed from the soil in grain or other harvested
products (wool, meat). Most WA soils are sandy so topsoils have low clay contents, and because of frequent cropping, low organic matter contents. The capacities of the soils to adsorb Zn are not well quantified, but relative to the small amounts of Zn applied as fertiliser, is probably adequate to sorb applied Zn. Regardless, WA soils hold most Zn in the top few cm of soil where the fertiliser was applied and incorporated into soil (Chapter 5; Barrow 1993). These are similar processes to those described by Barrow (1980) for the RV of fertiliser P. However, to understand the whole system, which determines the availability and RV of Zn the various, inputs and outputs of Zn in the soil-plant system need to be examined (Figure 7.1). The dominant factors affecting the residual effectiveness of Zn are (i) fertiliser reactions with soil (ii) leaching and erosion, (iii) removal in crop and livestock products, (iv) incorporation of Zn from fertiliser into plant shoot biomass and litter that recycle back to the soil organic matter pool and (v) addition of Zn to soil as fertilisers. The relative importance of each is discussed below.

**Removal in plant and livestock products:** The amount of Zn removed in product is typically low relative to the amount of Zn applied (Figure 7.1). In the present study (Chapter 6), where 3 kg Zn/ha had been applied to the soil in 1983, about 7% of the total Zn was removed in the grain from all the subsequent wheat and lupin crops in the 14 years up to 1996.

The pasture in the present study was not grazed or defoliated so negligible Zn was removed in the pasture years (see Section 7.3; Losses of Zn in pasture and animal systems). However, when grazing occurs removal of Zn in wool is a negligible loss (3.5 g/ha/yr) and in meat it is still low relative to grain (33 g/ha/yr). Although Zn loss from the soil system with the removal of cereal or lupin grain is small as described in this study, loss of Zn through the export of hay (oat, wheat), presently a large industry in medium to high rainfall areas of WA, could be larger. For example, a 10-12 t/ha cereal hay crop would remove about 300 to 360 g/ha (using Zn concentration of cereal hay from
Gartrell and Bolland 2000). This represents a 9-fold increase in Zn removal relative to that of grain and would greatly decrease the residual value of applied Zn (See Table 7.1).

In soils where hay harvesting is practised, annual Zn application (about 80 g Zn/ha) as a contaminant in superphosphate fall well short of replacing the removal (calculated using 150 kg/ha superphosphate with 600 mg Zn/kg).

![Figure 7.1](image.png)

**Figure 7.1** The soil-Zn system with possible Zn additions and losses to this system for soils of WA. The thickness of the arrow is an indication of the relative magnitude of the process.

*Leaching and erosion losses of Zn:* Losses of Zn from the soil system through leaching (see Chapter 5) are negligible because, as previously discussed, retention of Zn by soil, due to precipitation, adsorption and incorporation into organic matter, holds Zn close to where it was applied and incorporated into soil as fertiliser. The condition under which Zn leaching was examined in the present study represents a maximum risk scenario (acid
sandy soil and high rainfall) and since no leaching was detected, no leaching loss is shown in Figure 7.1.

**Erosion:** Soil erosion is the rapid removal of surface soil over a short period of time (Leeper and Uren 1993). As Zn is immobile in soil and remains in the top 5 cm of soil, processes such as wind or water erosion which remove all or part of this layer would result in Zn loss from the system. Large losses would result in a decline in the RV of previously applied fertiliser Zn necessitating a re-application of Zn fertiliser. Erosion loss of Zn could be calculated for such erosion events. The loss of Zn is equivalent to the concentration of Zn in the top surface soil multiplied by the weight of that section of soil lost. For example about 0.13 g Zn/ha will be lost if the surface 1-cm of soil (bulk density 1.3 g/cm³) is lost and it contained a Zn concentration of 1 mg/kg. However, management of vegetative cover by grazing and cropping strategies offers scope to prevent and control soil erosion. Most crops in WA are now sown using no-till and crop stubbles are retained greatly reducing soil erosion events (Reithmuller 2000). These practices in Australian agriculture have reduced erosion to negligible amounts (Leeper and Uren 1993). Therefore, most of the residual Zn is still retained in the soil under no-till cropping. No-till has really only become prevalent in the last 5-10 years, so before that erosion losses of Zn may have been greater.

**Reactions of fertiliser zinc with soil and root interception of the Zn fertiliser:** In the present study in Chapter 4.1, fertiliser Zn had been in contact with soil for up to 23 years, and in Chapter 6 for up to 14 years, and so had reacted with soil for a considerable time. The Zn was still effective and so still available to crops indicating a good RV. In addition, the Zn applied in previous years was increasingly incorporated throughout the soil when sowing crops with tined machines. Increased mixing (incorporation) of Zn through soil would have two effects:
1. The Zn would be mixed through a greater volume of soil so exposing the Zn to more adsorption sites on clay, sesquioxides, and organic matter in soil so increasing Zn retention by soil.

2. Increase the opportunity for interception of Zn in soil by plant roots growing through the soil so increasing Zn uptake by plant roots.

The net effect of decreased Zn availability and increased root interception of Zn in the soil is a decrease in effectiveness of Zn relative to freshly applied Zn for Zn uptake and production by plants (Takkar and Walker 1993).

The length of time Zn fertiliser is available in a soil (the residual value or RV) for maximum grain yields varies with soil properties, level of initial application of fertiliser Zn and the crop species since they differ in their ability to use soil Zn. For WA, the RV of Zn also varies with source of P fertiliser (Chapter 6). The present study showed that Zn applied to the soil had a RV greater than >20 years where superphosphate was used in the cropping rotation (see Chapter 4.1). However, the RV of Zn fertiliser was about 15 years where low additions of Zn were added (e.g. with the use of DAP) to the cropping rotation (see Chapter 6). As previously stated, studies done elsewhere have shown that the RV of Zn application is not as long lasting for other soil types and plant species (Takkar and Walker 1993). For example, Takkar et al. (1975) found that an application of 22 kg Zn/ha lasted 7 crops in a wheat-ground nut rotation on a loamy sand. Similarly, Weir and Holland (1980) measured a residual effect of 18 kg Zn/ha for at least 6 crops of maize in a single cropping rotation on a vertisol.

**7.2 Simple models for estimating the residual value of Zn**

The study of the separate processes of the Zn cycle in WA soils (see Figure 7.1) suggests that the cycle is relatively simple. Hence, a simple model for estimating the RV of Zn
fertiliser is possibly adequate for management of Zn status. Below three examples of spread-sheet-based decision support tools are presented as case studies in predicting the need for re-application of Zn fertiliser given different assumptions about yield, Zn applications and soil reactions.

7.2.1 Case 1-No further additions of Zn and irreversible reactions

Table 7.1. Case 1: The hypothetical number of crops with a range of possible grain yields that can be grown on a neutral yellow brown sandy loam from Newdegate where the recommended Zn application for the soil type (750 g Zn/ha) has been applied. Assumes no further additions of Zn as fertiliser or as an impurity in a fertiliser.

<table>
<thead>
<tr>
<th>Grains yield t/ha</th>
<th>Typical Zn concentration of grain (mg/kg)</th>
<th>Removal in grain (g/ha)</th>
<th>Zn fertiliser available for grain removal (g/ha)</th>
<th>Number of crops based on grain removala</th>
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<tbody>
<tr>
<td>1</td>
<td>22</td>
<td>22</td>
<td>525</td>
<td>23, 11</td>
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<td>2</td>
<td>22</td>
<td>44</td>
<td>525</td>
<td>11, 5</td>
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<td>3</td>
<td>22</td>
<td>66</td>
<td>525</td>
<td>7, 4</td>
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<tr>
<td>4</td>
<td>22</td>
<td>88</td>
<td>525</td>
<td>5, 3</td>
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<tr>
<td>5</td>
<td>22</td>
<td>110</td>
<td>525</td>
<td>4, 2</td>
</tr>
<tr>
<td>6</td>
<td>22</td>
<td>132</td>
<td>525</td>
<td>3, 2</td>
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</table>

aGartrell and Glencross (1968).
bMedian Zn concentration of wheat grain grown on Zn adequate sites of a neutral yellow brown sandy loam (n=26).
cAssumes soil reactions result in 70% (low sorption sandy soil) or 35% (high sorption clay soil) of the Zn in available forms for plant uptake currently or in the future (irreversible chemical reaction).
daThe number of crops rounded down to whole numbers. N.B. These values would also be the number of years if continuous cropping were practised.

In cropping systems of WA, Zn loss in grain is the major loss of Zn from the soil-Zn cycle. Based on the amount of Zn removed in grain, Case 1 (Table 7.1) illustrates the hypothetical maximum number of wheat crops that can be grown on low (yellow brown soil from Newdegate, WA) and high Zn sorption soils as a function of expected grain yield and soil Zn sorption. Case 1 assumes no further additions of Zn to the soil system either as Zn fertiliser or as an impurity of Zn in a macronutrient fertilizer and only cereals
Grain yields in WA are typically about 1.5 t/ha (Anderson et al. 2000; Tennant 2000). Based on this yield, the length of time Zn fertiliser would be available in the soil assuming weak Zn retention plus incorporation of Zn into soil organic matter by soils would be about 15 crops in Case 1 (Table 7.1). This agrees with data presented in Chapter 6. However, average wheat grain yields in WA are increasing due to the use of better varieties, improved management, higher soil fertility due to growing grain legumes or canola between cereals, retaining crop stubbles and reducing cultivation of soil to increase soil organic matter levels, higher use of fertiliser N for non-legume crops [cereals, canola] (Edward and Haagensen 2000). In these more productive systems, removal of Zn in grain would be higher than with 1.5 t grain/ha, reducing the number of crops for which the original Zn application sustains wheat grain yield. Similarly, if the Zn concentration of the seed was higher, the removal of Zn from the soil system would be significantly higher with the higher grain yield. That is, the RV of Zn fertiliser is lower and more frequent reapplication of Zn fertiliser would be required. In a more extreme example given in Table 7.1 where a larger proportion (about 70 %) of the applied Zn fertiliser was adsorbed (e.g. alkaline clay soil) and grain yields were 4 to 5 t/ha, Zn fertiliser would last about 2 to 3 crops. Similarly, if a greater proportion of the applied Zn was adsorbed than estimated by the incubation technique of Chapter 2, and grain yields were 2 t/ha, only 5 crops could be grown before Zn fertiliser needed to be re-applied (Table 7.1). However, as reported in Chapter 6 re-appearance of Zn deficiency after 2 to 5 crops has not been observed under the current agricultural farming systems of WA because of Zn-contaminated superphosphate.

Presently, in medium to high rainfall areas of WA where cereal yields are about 4 to 5 t/ha (Tennant 2000; Hill and Carslake 2003; Zhang et al. 2004) Zn deficiency has not appeared as a problem for crop yield within the past 10 years since Zn fertiliser
application. However, the calculated RV in the hypothetical example (Table 7.1) suggests that Zn-deficiency would be observed within about 4 cereal crops (about 8-12 years).

Evidently, the RV of fertiliser Zn in the medium and high rainfall areas is much greater than the calculated values based on the assumptions used in Table 7.1. Therefore it is necessary to re-examine the assumptions in Table 7.1 that reactions of applied Zn with soil were irreversible, and there are no further additions of Zn as a fertiliser or as a contaminant in macronutrient fertilisers.

7.2.2 Case 2  Further additions of Zn as a contaminant and irreversible reactions

From Table 7.1 it is suggested that after about 11 crops (assuming 2 t cereal grain/ha) the Zn applied in fertiliser would be depleted. However, it is common practice in WA to add 600 mg Zn/kg to superphosphate and ammonium phosphate fertilisers manufactured in WA to maintain the Zn status of soils. Therefore, RV measured in the field is frequently longer (see Chapter 6) than that determined from Table 7.1. Consequently, in Case 2 (Table 7.2) it is assumed the superphosphate or ammonium phosphate fertiliser applied to the experiment contains 600 mg Zn/kg. As for case 1, irreversible reactions of applied Zn with soil are assumed in the soil-plant Zn model.

Increasing grain yields due to more productive systems results in the need to supply larger amounts of fertiliser P and N to sustain the larger crop yields. If the P or P and N fertilisers are contaminated with Zn, or Zn is added to the fertilisers during manufacture to maintain 400-600 mg Zn/kg fertiliser, then more Zn will be applied to the crops as the amounts of NP fertiliser applied to the crops increases. For cereal grain yields of less than 2.0 t/ha about 10 kg P/ha is typically required to supply sufficient P. For grain yields between 2 to 4 t/ha about 12 kg P/ha is required (Bolland pers. comm.). However, for higher grain yields >5 t/ha about 15 kg P/ha would be required. Therefore, these three levels of P addition are used in Table 7.2 to calculate the amount of Zn as a contaminant
added to soil together with the P fertiliser applied to the crop.

**Table 7.2: Case 2.** The hypothetical number of crops with a range of possible grain yields that can be grown on a neutral yellow brown sandy loam from Newdegate where the recommended Zn application for the soil type (750g Zn/ha) has been applied. Assumes further additions of Zn as an impurity in superphosphate and ammonium phosphate fertiliser applied to provide P and N to the crop.

<table>
<thead>
<tr>
<th>Grain yield t/ha</th>
<th>Typical Zn conc. of grain (mg/kg)</th>
<th>Addition (g Zn/ha) in fertiliser</th>
<th>Removal (g/ha) in grain</th>
<th>Surplus (+) or deficit (-)</th>
<th>Zn fertiliser available for grain removal</th>
<th>Number of crops based on crop removal</th>
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<tr>
<td>1</td>
<td>22</td>
<td>60(^b)</td>
<td>22</td>
<td>+38</td>
<td>525</td>
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<td>2</td>
<td>22</td>
<td>60</td>
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<td>+16</td>
<td>525</td>
<td>70 % 35 %</td>
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<tr>
<td>3</td>
<td>22</td>
<td>72</td>
<td>66</td>
<td>+6</td>
<td>525</td>
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<td>4</td>
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<td>72(^i)</td>
<td>88</td>
<td>-16</td>
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<td>70 % 35 %</td>
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<td>32(^j) 16</td>
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\(^a\)Gartrell and Glencross (1968).  
\(^b\)Median Zn concentration of wheat grain grown on Zn adequate sites of a neutral yellow brown sandy loam (n=26).  
\(^c\)Assumes the Zn is in available forms for plant uptake currently or in the future.  
\(^d\)The number of crops not the number of years unless continuous cropping practised.  
\(^e\)Based on the residual effectiveness of soil 13 as previously determined (Chapter 5)  
\(^f\)Assumed extreme “fixation” of applied Zn.  
\(^g\)Based on addition of superphosphate at 10 kg P/ha (Bolland per. com.) containing Zn at 600 mg/kg  
\(^h\)The symbol “+” indicates that Zn is increasing in the soil system.  
\(^i\)Based on addition of superphosphate at 12 kg P/ha and Zn at 600 mg/kg  
\(^j\)Number of crops rounded down to the lowest whole number.  
\(^k\)Based on addition of superphosphate at 15 kg P/ha and Zn at 600 mg/kg.

In case 2, when an average grain yield of about 1.5 t/ha is assumed within the model, the additions of Zn are greater than the losses of Zn from the system. In fact, it is not until grain yields reach about 3.25 t/ha that the loss of Zn in grain equals the addition of Zn to the soil-plant system from the fertiliser. That is, application of superphosphate or ammonium phosphate fertilisers containing Zn at 600 mg/kg of fertiliser result in a net increase in the Zn status of the soil, where grain yields are 3 t/ha or less. Therefore, as the result of applying fertilisers contaminated with Zn and assuming most of the added Zn remains plant available the Zn status of the soil remains adequate for all crop species.
grown in the soil. This result supports data presented in Chapter 4.1 where fertiliser Zn had a very long RV (>20 years) when superphosphate containing about 600 mg Zn/kg of fertiliser was applied annually at 150 kg/ha. However, in higher rainfall regions, and with the continual improvement in management of cropping in all rainfall zones, the grain yield of crops will improve resulting in higher amounts of Zn removed in grain. The removal of Zn in grain as grain yields increase will reduce the RV of Zn for maximum grain yield. From Table 7.2, even where grain yields are about 6 t/ha, the RV of Zn fertiliser would be about 10 crops. Currently, in medium to high rainfall areas (>350 to <750 mm mean average rainfall) where grain yields are 4 to 5 t/ha, Zn deficiency is not apparent or widespread even after 5 to 10 crops. This suggests the RV of the previously added Zn is still currently adequate to avoid Zn deficiency of cereals. This may mean that higher yielding crops produce a greater amount of roots to explore greater volumes of moist soil at depth and so intercept sufficient Zn in moist soil to avoid deficiency. It is therefore concluded that for systems aimed at maximising grain yields in all rainfall zones, addition of the recommended amount of Zn to the first crop together with applying N and P fertiliser enriched with Zn to subsequent crops have both maintained the Zn status of most WA soils. This practice has prevented widespread Zn deficiency reducing grain yields. From Table 7.2, it therefore appears that, provided the recommended amount of Zn has been applied to the first crop (see Gartrell and Glencross 1968), adding single superphosphate or compound fertilisers contaminated with 600 mg Zn/ha at amounts greater than 150kg/ha to subsequent crops will maintain the Zn status of most WA soils used for agriculture. For high yielding crops of 4 to 6 t grain/ha, the RV of Zn fertiliser ranged from 32 to 12 years for these grain yields and fertiliser inputs (Table 7.2).

The Zn contamination of other fertilisers typically used in WA agriculture is low. Urea is widely used as an N fertiliser and typically contains about 20 mg Zn/kg of urea. Urea is usually applied at 70-100 kg urea/ha, supplying only 1.4 to 2.0 g Zn/ha or about 1-3% the
Zn present in Zn-contaminated superphosphate. Potassium chloride, (muriate of potash, KCl) is increasingly being used in WA agriculture, and the fertiliser typically contains about 50 mg Zn/kg of KCl. As KCl is usually applied at about 75 to 100 kg/ha, about 3.5 to 5.0 g Zn/ha is applied. Therefore, the application of both urea and KCl would supply small amounts of Zn to the soil that represents the amount of Zn removed in about 100 to 200 kg of cereal grain. The triple superphosphate (TSP) used in WA agriculture is imported and typically contains 400 mg Zn/kg of TSP. As TSP contains about 20 % P, double the amount of P as superphosphate, the TSP is usually applied at about half the rate as superphosphate which results in the application of 60-70 % less Zn than Zn contaminated or enriched superphosphate with 600 mg Zn/kg superphosphate.

Cases 1 and 2 assumed irreversible retention of applied Zn by reactive soil constituents. However, small amounts of Zn incorporated into organic matter or adsorbed onto soil are likely to be mineralised or desorbed to provide water-soluble Zn in soil solution that can be taken up by plant roots growing in moist soil. Hence, sorption of applied Zn is unlikely to be completely irreversible. However, the amounts of Zn in soil solution are extremely small, and most soil Zn is in an insoluble organic or inorganic form (Figure 7.1). Case 2 closely resembles the RV of fertiliser Zn applied (Chapter 4.1) for cropping systems in WA where small additions of Zn have resulted in a long RV of Zn fertiliser.

7.2.3 Case 3 Additions of Zn, and irreversible reactions and removal in rotations

Cases 1 and 2 have dealt with the simple case of a single crop and its capacity to deplete soil Zn. Case 3 illustrates the two extremes for a typical cropping rotation in farming systems within WA by using firstly, average current yields and then secondly, twice the average.

About 13g Zn/ha is lost from the soil-plant cycle under the low production system (Table 7.3a), while about 115 g Zn/ha is lost under the more productive system (Table 7.3b) with no Zn added in the cropping rotations. Compared to continuous cropping, the pasture
phase has a considerable sparing effect on Zn depletion. By contrast, the crops while varying in yield and Zn concentration in seed have relatively similar Zn uptake.

Table 7.3: Case 3. The hypothetical number of crops that can be grown in a pasture (P), wheat (W), lupin (L) and canola rotation at two yield levels on a neutral yellow brown sandy loam from Newdegate where the recommended Zn application (750 g Zn/ha) has been applied. Assumes further additions of Zn as an impurity in superphosphate supplied to the pasture phase and negligible Zn in the ammonium phosphate fertiliser applied to provide P and N to the crops. The amount of Zn available in the soil is based on the residual effectiveness (RE) for the soil type (Chapter 5).

(a) Soil Zn cycling at average yield of wool (P), wheat (W), lupin (L) and canola (C) in WA

<table>
<thead>
<tr>
<th></th>
<th>Yield (t/ha)</th>
<th>Zn in Produce (mg/kg)</th>
<th>Zn removed (g/ha)</th>
<th>Zn added (g/ha)</th>
<th>Zn balance (g/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasture: clean wool</td>
<td>0.035</td>
<td>110</td>
<td>3.5</td>
<td>90</td>
<td>86.5</td>
</tr>
<tr>
<td>Wheat</td>
<td>1.5</td>
<td>22</td>
<td>33</td>
<td>0</td>
<td>-33</td>
</tr>
<tr>
<td>Lupin</td>
<td>1.1</td>
<td>30</td>
<td>33</td>
<td>0</td>
<td>-33</td>
</tr>
<tr>
<td>Canola</td>
<td>1.1</td>
<td>30</td>
<td>33</td>
<td>0</td>
<td>-33</td>
</tr>
</tbody>
</table>

Total -12.5

Zn (g/ha) for soil type = 750

RE for soil type = 0.7

No of cycles of P:W:L:C = 42

years = 168

(b) Soil Zn cycling at twice average yield of wool, wheat, lupin and canola in WA

<table>
<thead>
<tr>
<th></th>
<th>Yield (t/ha)</th>
<th>Zn in Produce (mg/kg)</th>
<th>Zn removed (g/ha)</th>
<th>Zn added (g/ha)</th>
<th>Zn balance (g/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasture: clean wool</td>
<td>0.07</td>
<td>110</td>
<td>7</td>
<td>90</td>
<td>83</td>
</tr>
<tr>
<td>Wheat</td>
<td>3.0</td>
<td>22</td>
<td>66</td>
<td>0</td>
<td>-66</td>
</tr>
<tr>
<td>Lupin</td>
<td>2.2</td>
<td>30</td>
<td>66</td>
<td>0</td>
<td>-66</td>
</tr>
<tr>
<td>Canola</td>
<td>2.2</td>
<td>30</td>
<td>66</td>
<td>0</td>
<td>-66</td>
</tr>
</tbody>
</table>

Total -115

Zn (g/ha) for soil type = 750

RE for soil type = 0.7

No of cycles of P:W:L:C = 4.565

years = 18

The Zn balance is equal to the addition less the amount of Zn removed in the produce. The symbol ‘-‘ means a net loss of Zn from the soil system.

Gartrell and Glencross (1968).

The RE of the soil based on data from Chapter 5.

The number of rotation cycles calculated from the multiplication of the recommended Zn level by the RE for the soil type divided by the total Zn balance for a cycle.

The number of years is equivalent to the multiplication of the number of rotation cycles and the number of crops in the cycle.
(Gartrell and Glencross 1968) could all vary from site to site resulting in significant variations in RV of Zn fertiliser. A simple Zn budgeting of the form used in Case study 3 can account for these factors and would be a useful tool for Zn fertiliser use. A complete model of Zn cycling is not justified as discussed above. Tables with ranges of input values from the literature are available and these could be used in the spreadsheet. These values could be checked with soil and plant analysis to redefine the values used in each farm situation. Values for a particular soil could be entered (shaded grey scale in Table 7.3) and then the number of rotation cycles would be calculated.

The output is the number of years that Zn fertiliser remains effective for maximum agricultural production. In the example of Table 7.3, the RV of Zn would be about 168 years in the low productivity compared to about 18 years (Table 7.3b) in the higher productivity system of WA. Numerous combinations of additions of Zn and losses of Zn in grain could be made. However, data presented in this work (Chapter 4 & 6) suggests that the RV of Zn fertiliser for each individual farming system used in WA is between these extremes illustrated above. Further considerations of factors affecting the RV are outlined in Chapter 7.3.

7.3 Further factors affecting the residual value of Zn

Zinc in plant herbage is returned to the soil as Zn complexed with organic matter and many organic compounds (Stevenson and Ardakani 1972; Stevenson 1991). Soil organisms process the organic matter and organic compounds and the Zn re-enters the soil-Zn cycle. Zinc cycling in the soil-plant-animal system is a process not well documented in the literature. The Zn in herbage harvested as pasture hay is either returned to the Zn system via animal feeding or removed if the produce is exported off the farm. For example, pasture hay yielding 7 t/ha would remove about 175 g Zn/ha if completely removed from the farm (calculated from pasture Zn levels in WA from
The Zn in herbage ingested by animals either returns to the soil-Zn system via excreta or removed in produce (meat, wool, and milk). The amounts removed in the produce of animals are typically low relative to the amount of fertiliser Zn applied. In animals, the whole body concentration of Zn on a fresh basis range from 20 to 30 mg/kg in pigs (Sprage and Widdowson 1950), cows (Miller et al. 1974) and sheep (Grace 1983). Zinc appears to be evenly distributed through the animal body and on a dry weight basis ranges from 20 to 250 mg/kg (Underwood 1977, O’Dell 1979, Hill et al. 1983a, b, c). Generally, the highest concentration of Zn is in bone, liver, pancreas, kidney and muscle. About 2 to 3% of Zn in cattle is found within the skin and hair. However, for sheep about 55% of the body Zn can be in wool. Masters and Moir (1980) found about 110 mg Zn/kg in wool of sheep from WA. Based on wool production of 60 to 80 kg/ha during the spring (August to November) in an intensively grazed pasture system of WA (Thompson et al. 1994, 1997; Hyder et al. 2002) and using a wool Zn concentration of 110 mg/kg (Masters and Moir 1980) Zn loss ranges from about 6.5 to 8.8 g/ha. Similarly, 50 kg mature Merino wethers grazed at 12 sheep/ha (Thompson et al. 1997) and containing 30 mg Zn/kg body mass (Grace 1983) would remove about 1.8 g Zn/ha from an intensive grazed pasture system in WA. Hence, the loss of Zn from an intensively grazed system assuming complete removal of the animals and wool (about 8 to 10 g Zn/ha) from the system represents about 1 to 1.5% of the amount of Zn applied as a fertiliser in agricultural systems of WA. In set stocking-rate grazing systems (Thompson et al. 1994, 1997; Hyder et al. 2002), the loss of Zn from the system would be about one third to half that calculated for the intensively grazed system. The set stocking-rate grazing system would probably be the more typical case study for WA (Hyder pers comm.). Hence the set stocking-rate grazing system should remain adequately supplied with Zn almost indefinitely after an initial application of ZnO.

The complicating and exacerbating effects of herbicides, which reduce the uptake of Zn
by roots of plants, could further affect the RV of Zn fertiliser. In soils that have a marginal or deficient supply of Zn, the applications of diclofop-methyl and chlorsulfuron have been found to intensify Zn deficiency in wheat (Robson and Snowball 1989; 1990). The effect of herbicides is through changes in root morphology of the plant, commonly called “root pruning”. The “root pruning” effect of these herbicide alters the total volume of soil through which the roots grow (less root exploration of the soil), and decreases the amount of soil Zn that roots can contact (McLay and Robson 1992; Osborne and Robson 1992; Osborne et al. 1993; Nable and Webb 1993; O’Keeffe and Wilhelm 1993). Hence as residual value of Zn declines, Zn deficiency may re-appear earlier when the sulfonyl-urea herbicides are used for weed control, requiring more frequent re-application of Zn than suggested above (e.g. Table 7.3). The root pruning effect of various herbicides on Zn uptake has been confirmed as particularly important in cereal growing areas of low plant available Zn (O’Keeffe and Wilhelm 1993). In Australia, large areas of the cereal growing areas have been identified as having low plant available Zn (Sillanpää and Vlek 1985), and therefore cereal crops grown in these particular areas may potentially suffer grain yield losses when sulfonyl-urea herbicides are used. By contrast, these herbicides are not used for weed control in legume and oilseed crops in the rotation (Pritchard and Carpenter 1993; Littlewood 2003). The decreased contact with soil Zn as a result of changes in root morphology by herbicides is opposite to the effect of the VAM mycorrhizae where the fungi hyphae extend the root system and increase the amount of soil-Zn contact (Thompson 1990; Ryan and Angus 2003). Abbott and Robson (1991) have demonstrated that VAM mycorrhizae are present in many soils of WA. However, as the level of P fertiliser applied to soil increases, root colonisation of AM mycorrhizae decreases (Singh et al. 1986; Abbott et al. 1995). Therefore on soils with low Zn supply, a depression in mycorrhizal activity in roots is often associated with a decrease in Zn concentration of shoots (Singh et al. 1986). Marschner (1993) suggested that a decrease
in Zn concentration may not be evident where P fertiliser contains substantial amounts of Zn as for the P fertilisers of WA.

The use of Zn-efficient genotypes is considered a practical approach for cropping on soils of low Zn status (Graham et al. 1992; Cakmak et al. 1999), especially in regions where Zn fertilisers are expensive and not always effective in eliminating Zn deficiency (Genc and Donald 2004). Nutrient efficiency is defined as the ability of a genotype to grow and produce maximum yield in soils Zn deficient for a standard genotype (Graham 1984). Differences in Zn requirement of plant species as well differences between genotypes would affect the RV of Zn by either (i) increasing the RV for efficient plant species and genotypes, or (ii) decreasing the RV for inefficient plant species and genotypes. The selection of Zn-efficient plant species and genotypes would extend the RV of Zn in soils of WA.

7.4 Some limitations of this research and suggestions for future work.

In this study incubation of added Zn in moist soil has been used to simulate soil reactions that decrease levels of plant available Zn (Chapter 2). Several limitations of the moist incubation of Zn technique for the study of RV of Zn are: (i) the length of the time and temperature at which the Zn was incubated with the soil and (ii) the relationship between the incubation technique and Zn reactions in soil in the field situation.

Varying the length of time over which the incubation took place and the temperature of the incubation were not studied. The time period used for the incubation may not have allowed all Zn reactions to be completed, although similar time periods have been used in studying the RV of Cu (Brennan et al. 1980; 1984). However, Brennan et al. (1980; 1984) showed that both the temperature and length of time for incubation of Cu with moist soil was important in determining the RV of Cu for wheat. Therefore, a similar study on the effects of temperature and length of the incubation could determine the role
these two parameters have on the RV of Zn fertiliser. This proposed study would allow
the reactions between Zn and properties to reach completion. Although the relative
differences among soils may possibly not change, the absolute amount of Zn available for
plant uptake would likely decline, affecting the RV of Zn.

Another major limitation is that for the incubation study, added Zn was homogeneously
mixed through a finite volume of soil that is maintained moist unlike the conditions of soil
used for growing crops in the field. The distribution of Zn applied in the field is likely to
be much more heterogeneous in the top 10 cm of soil in the field. In addition, the surface
soil would intermittently dry between rainfall events during the growing season, and plant
uptake of nutrient elements from dry soil is greatly diminished (Wilkinson 1972). The
RV determined in the glasshouse is more likely to represent the RV determined in field
experiments done in high rainfall areas when applied Zn is incorporated into the top 10
cm of soil by multiple-soil cultivations while killing weeds and sowing crops over several
years (Reithmuller 2000). Furthermore, 60 % of WA crops are now sown and fertilised
using no-till, in which stubble of previous crops is retained, weeds are controlled with
herbicide sprays, and soils are only cultivated in narrow rows 23 to 30 cm apart to sow
seed in narrow slots 2 to 5 cm deep. With no-till, any fertiliser Zn placed (drilled) with
the seed while sowing is likely to remain where it was applied in bands 23 to 30 cm apart
and 2 to 5 cm deep. This would reduce root interception of Zn applied by no-till, and the
lack of subsequent soil cultivation would likely reduce the RV of the recently-applied Zn.
Furthermore, in no-till systems the amount of Zn retained in the stubble would increase
before microbial action would release this source of Zn into the soil surface where its
availability for plant uptake is limited. In tillage systems prior to no-till, the stubble was
burnt, the Zn content of the stubble immediately released, and ploughing into the soil
meant that Zn was readily available for plant uptake. These aspects of no-till systems on
the RV of Zn require further research.
In the field work of this study, the RV was measured using the oxide source of Zn. There are several Zn compounds that can be used as fertilisers. Mortvedt and Gilkes (1993) divided the sources of Zn into four main groups: inorganic, synthetic chelates, natural organic complexes, and inorganic complexes. Inorganic sources include Zn oxide (this study), Zn sulfate, Zn carbonate, Zn nitrate, and Zn chloride. Zinc sulfate is the most common source and sold as crystalline and granular forms (Mortvedt and Gilkes 1993). Zinc oxide has low effectiveness in the granular form as it is insoluble in water (Mortvedt 1991). Water solubility of the Zn source is considered important and it has been suggested about 50 % water solubility is required for the Zn source to be effective for calcareous soils (Westfall et al. 1999; Amrani et al. 1999; Gangloff et al. 2002; Westfall et al. 2002). This appears to conflict with the historic use of the oxide source in WA, where early researchers of Zn nutrition (Toms 1958; Gartrell and Glencross 1968 and others) found that the oxide and sulfate source were equally effective (unpublished data of Dept. Agric.; pers. comm. JW Gartrell). However, the ZnO was finely ground and dry mixed with superphosphate. The sulfate source was granular; hence the results of the comparison work may have been confounded by differing particle size of the Zn sources. However, in acid soils, a diverse range, including the oxide and sulfate, are equally effective Zn sources provided the sources are fine powders and mixed through the soil (Mortvedt and Gilkes 1993). Water insoluble ZnO and soluble Zn sulfate are equally effective when incorporated into granules of superphosphate (Gartrell and Glencross 1968). About 75 % of the approximate 18 million ha used for agriculture in WA are acidic to neutral; the remaining 25 % are neutral to alkaline. Further glasshouse studies are required in WA to compare the effectiveness of finely ground Zn oxide (present source) with finely ground and crystalline and granular Zn sulfate for previously unfertilised typical acidic and alkaline soils in the region. Unfertilised soils exist under remnant vegetation still present in the region and have never been fertilised.
The RV of fertiliser Zn is measured in large long-term trials that are expensive to maintain so only a limited number of field experiments can be undertaken. So the field data on RV are confined to few soil types, environments, years (seasons) and management systems used in the limited number of field experiments. This data can be extended to other soils, environments, years, crop species and cropping systems now used in WA agriculture by the use of detailed glasshouse studies to determine RV for a range of different soil types. Subterranean clover and wheat crops have been the major crops used in glasshouse and field studies (Chapter 3 & 4). In this study additional information on the relative Zn uptake of lupin, canola, faba bean, chickpea, lentils and durum has been obtained. There are no data for the new pasture crops now grown in WA (serradella, annual medic, biserrulla pasture legumes crops), requiring further research. Within each crop and pasture species there are likely to be cultivar differences in uptake of Zn (Graham et al. 1992; Graham and Rengel 1993; Cakmak et al. 2001; Erenoglu et al. 1999) that would need to be accounted for in the model on the RV of Zn fertiliser. However, the significance of cultivar differences in Zn efficiency for RV of Zn fertiliser has not been researched.

Canola, although non-mycorrhizal, has fine roots and abundant root hairs (Brewster et al. 1976). For example, under P-deficient conditions, the root hairs increase in length and density (Foehse and Jungk 1983) enabling canola roots to explore a greater volume of soil thereby increasing P uptake by the roots. In addition, canola roots acidify the rhizosphere just behind the root tips, dissolving insoluble forms of P in the soil and increasing P uptake from the soil (Grinsted et al. 1982; Hedley et al. 1982; Moorby et al. 1988; Hoffland et al. 1989a, b). The role of fine roots and rhizosphere acidification on the availability of Zn has not been fully investigated. However, Marschner et al. (1987) have found that rhizosphere acidification has increased the availability of Fe for uptake by plants. The release of carboxylates from the roots of plants mobilises bound P that is
unavailable for plant species that do not release carboxylates (Veneklaas et al. 2003). Plant species vary in the amounts and forms of carboxylates that are released. Generally higher amounts of carboxylates are released under low P supply (Pearse et al. 2003). Possibly Zn availability would be increased by rhizosphere acidification and/or by carboxylate release. However, direct evidence of a beneficial role of rhizosphere acidification and/or carboxylate release on Zn uptake is still lacking except for those studies on rice which have limited relevance to the crops and soils of interest in this thesis.

Changes in soil Zn fractions under intensive cropping rotations involving cereals, grain–legumes and canola have not been measured in WA. With the increase in intensity of cropping and the shift to no-till operations, it would be prudent to establish long-term large scale field experiments where the soil fractions of Zn could be monitored over 7 to 15 years. Soil samples from several soil depths (0-10, 10-20, 20-30 cm) could be collected at the commencement of the cropping rotation and after several cycles of the rotation. These soil samples could be analysed for both total and extractable Zn. Inputs and losses of Zn from the soil system would need to be monitored. This does not appear to have been done for broad-scale agricultural crops. However, a similar soil-sampling technique for Zn has been used in intensive silviculture to measure the productivity of radiata pines (Boardman 1982; 1986). Such effects of induced Zn deficiency in intensive silviculture were noted in vigorous growing radiata pines following the application of N and P fertiliser (Boardman and McGuire 1990).

In conclusion, this thesis has culminated in a better understanding of Zn in the agricultural production systems of WA. The distribution and correction of Zn deficiency is now predictable for the many soil types and cropping systems of WA. Accurate identification of Zn deficiency for a range of crop and pasture species by plant analyses, typically the youngest mature leaf, is now possible. With the calibration of the DTPA Zn soil test for
soils of WA, particularly for wheat the major crop species grown in WA, prognosis of potential Zn deficiency can now be predicted before the appearance of Zn deficiency or loss in plant production. Understanding of the soil properties that influence the availability of Zn and reduce the RV of Zn in WA soils has been greatly improved. Spreadsheet models were developed to determine when re-application of fertiliser Zn was required for low and high production systems of WA.
Appendix 1.

The location, description (texture, colour), percentage sand and cation exchange capacity (CEC) of the soils used in glasshouse work of Chapter 2 & 3
<table>
<thead>
<tr>
<th>No.</th>
<th>Location*</th>
<th>Description</th>
<th>Texture</th>
<th>Colour</th>
<th>Sand</th>
<th>CEC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Busselton</td>
<td>Mungite grey sand</td>
<td>S</td>
<td>G</td>
<td>97.0</td>
<td>7.4</td>
</tr>
<tr>
<td>2</td>
<td>Badgingarra</td>
<td>Grey sand</td>
<td>S</td>
<td>G</td>
<td>98.0</td>
<td>1.8</td>
</tr>
<tr>
<td>3</td>
<td>Newdegate</td>
<td>White sand</td>
<td>S</td>
<td>W</td>
<td>96.0</td>
<td>1.7</td>
</tr>
<tr>
<td>4</td>
<td>Wongan Hills</td>
<td>Grey sandy loam</td>
<td>SL</td>
<td>G</td>
<td>94.0</td>
<td>1.2</td>
</tr>
<tr>
<td>5</td>
<td>Wyalkatchem</td>
<td>Gritty grey sand</td>
<td>S</td>
<td>G</td>
<td>93.0</td>
<td>1.6</td>
</tr>
<tr>
<td>6</td>
<td>Busselton</td>
<td>Mungite grey sand loam</td>
<td>SL</td>
<td>Y</td>
<td>95.0</td>
<td>3.1</td>
</tr>
<tr>
<td>7</td>
<td>Esperance</td>
<td>Fleming grey sand</td>
<td>S</td>
<td>G</td>
<td>97.0</td>
<td>1.9</td>
</tr>
<tr>
<td>8</td>
<td>Sth. Australia</td>
<td>Deep Sand</td>
<td>S</td>
<td>G</td>
<td>96.0</td>
<td>1.5</td>
</tr>
<tr>
<td>9</td>
<td>Lancelin</td>
<td>Brown sand</td>
<td>S</td>
<td>B</td>
<td>96.0</td>
<td>2.4</td>
</tr>
<tr>
<td>10</td>
<td>Jerramungup</td>
<td>Grey sand</td>
<td>S</td>
<td>G</td>
<td>95.0</td>
<td>2.4</td>
</tr>
<tr>
<td>11</td>
<td>Busselton</td>
<td>Mungite grey sandy loam</td>
<td>SL</td>
<td>G</td>
<td>94.0</td>
<td>4.1</td>
</tr>
<tr>
<td>12</td>
<td>Moora</td>
<td>Yellow-brown sand</td>
<td>S</td>
<td>YB</td>
<td>96.0</td>
<td>1.6</td>
</tr>
<tr>
<td>13</td>
<td>Newdegate</td>
<td>Yellow-brown sandy loam</td>
<td>SL</td>
<td>YB</td>
<td>94.0</td>
<td>3.2</td>
</tr>
<tr>
<td>14</td>
<td>Pemberton</td>
<td>Karri SL</td>
<td>SL</td>
<td>Y</td>
<td>88.0</td>
<td>2.2</td>
</tr>
<tr>
<td>15</td>
<td>Kununurra</td>
<td>Cockatoo Sand</td>
<td>S</td>
<td>R</td>
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<td>2.1</td>
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<td>Redmond</td>
<td>Plantageant peaty sand</td>
<td>PS</td>
<td>BL</td>
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<td>6.4</td>
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<td>Acid sand</td>
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<td>6.8</td>
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<tr>
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<td>Tinnindewa</td>
<td>Yellow-brown sand</td>
<td>S</td>
<td>B</td>
<td>94.0</td>
<td>1.5</td>
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<tr>
<td>19</td>
<td>Talbot Brook</td>
<td>Brown gravelly sand</td>
<td>GS</td>
<td>B</td>
<td>90.0</td>
<td>4.8</td>
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<td>20</td>
<td>York</td>
<td>Grey sand</td>
<td>GS</td>
<td>B</td>
<td>90.0</td>
<td>4.8</td>
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<td>W</td>
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<td>L</td>
<td>B</td>
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<td>10.0</td>
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<td>R</td>
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<td>Brown sandy loam</td>
<td>SL</td>
<td>Y</td>
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<td>12.0</td>
</tr>
<tr>
<td>26</td>
<td>Salmon gums</td>
<td>Circle valley sand</td>
<td>S</td>
<td>W</td>
<td>93.0</td>
<td>5.1</td>
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<td>Yuna</td>
<td>Yellow sand</td>
<td>S</td>
<td>Y</td>
<td>94.0</td>
<td>1.4</td>
</tr>
<tr>
<td>28</td>
<td>Dandaragan</td>
<td>Fine red sand</td>
<td>S</td>
<td>R</td>
<td>93.0</td>
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<td>BL</td>
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<td>G</td>
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<td>BL</td>
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</tbody>
</table>

*aLocation for WA is the closest town or district name. For soils from other parts of Australia the state name is given..
Description are local names for WA soils and for the soils from other parts of Australia the description used was as that supplied.

Texture as classified McArthur (1991). S is sand; SL is sandy loam; C is clay, LS is loamy sand; PS is peaty sand; GS is gravelly sand.

The colour determined in the moist state (McArthur 1991). G is grey; w is white; YB is yellow-brown; RB is red-brown; Bl is black; B is brown; R is red and Y is yellow.

Sand content by the methods of Day (1965).

CEC is the cation exchange capacity determined by the methods of Tucker (1974).
Appendix 2.

The correlation matrix (r) for the soil properties of a range of soils used in Chapter 2.
<table>
<thead>
<tr>
<th></th>
<th>pH&lt;sub&gt;Ca&lt;/sub&gt;&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Sand&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Silt&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Clay&lt;sup&gt;b&lt;/sup&gt;</th>
<th>OC&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Al&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
<th>Fe&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
<th>CaCO&lt;sub&gt;3&lt;/sub&gt;&lt;sup&gt;e&lt;/sup&gt;</th>
<th>CEC&lt;sup&gt;f&lt;/sup&gt;</th>
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<td></td>
<td></td>
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<tr>
<td>Sand</td>
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<tr>
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<td>0.1047</td>
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<tr>
<td>Al&lt;sub&gt;2&lt;/sub&gt;O&lt;sub&gt;3&lt;/sub&gt;</td>
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<td>-0.1685</td>
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<td>0.1357</td>
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<td>0.1451</td>
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</table>

<sup>a</sup>pH<sub>Ca</sub> is the soil pH value measured in CaCl<sub>2</sub> (Rayment and Higginson 1991).

<sup>b</sup>Sand, silt and clay percentage content is the textural fractionation (Day 1965).

<sup>c</sup>The organic carbon content (Walkley and Black 1934).

<sup>d</sup>Fe<sub>2</sub>O<sub>3</sub> and Al<sub>2</sub>O<sub>3</sub> is the iron (Fe) and aluminium (Al) content (Hesse 1971).

<sup>e</sup>CaCO<sub>3</sub> is the calcium carbonate content (Rayment and Higginson 1991).

<sup>f</sup>CEC is the cation exchange capacity (Tucker 1974).
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