Changes with Time in the Availability of Soil Applied Zinc to Navy Beans and in the Chemical Extraction of Zinc from Soils

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Abstract

The effect of the incubation of zinc (Zn) applied to the soil on Zn uptake and the Zn concentrations in chemical extractants was studied. In a glasshouse experiment using a Zn-deficient gravelly sandy loam, the effect of recently applied Zn was compared with that of Zn incubated with the soil for 15 days at 40°C on growth and Zn uptake by navy beans (*Phaseolus vulgaris* cv. Gallaroy). At the second harvest (33 days after sowing), the dry weight of shoots of recently applied Zn was consistently higher than that of incubated Zn, except at the highest rate of $1 \mu g \, Zn \, g^{-1}$ soil, where yields were similar. Comparisons of the slope of the linear regressions of Zn uptake as a function of rate of application showed that incubated Zn was approximately 80% as effective as recently applied Zn.

A laboratory experiment measured the decrease in Zn concentration in HCl, EDTA, DTPA, and dilute $CaCl_2$ with incubation for up to 8 days at 40°C in four contrasting soils from Western Australia and Queensland. An addition of $2 \cdot 5 \mu g \, Zn \, g^{-1}$ soil increased the concentration of Zn in all extractants at all times of incubation compared with the untreated soil. The recovery of the added Zn was generally highest with HCl and lowest with $0 \cdot 002$ M $CaCl_2$ and decreased exponentially in all extractants with increasing time of incubation in all soils. The order of the rate of decrease in Zn concentration for all extractants was krasnozem > gravelly sandy loam > sand > sandy clay loam. The model, $Y = Ct^B$, where C and B are constants, was used to describe the relationship between the recovery of added Zn and time of incubation.

Introduction

Zinc (Zn) deficiency occurs in a variety of crops grown on a wide range of Australian soils (Donald and Prescott 1975). As a result, Zn is applied to large areas of soil to increase productivity of crops and pastures. Reactions of Zn with soil constituents appear to be the major factor determining decreases in availability of applied Zn to plants because losses of Zn by crop removal (Mengel and Kirkby 1978) and by leaching (Brennan and McGrath 1988) appear to be minor.

There is some evidence that Zn availability to plants decreases with time after application to the soil. Brennan and Gartrell (1986) reported that the yield of subterranean clover grown with Zn incubated for 30 days at 30°C was as low as 62% of that obtained with a fresh Zn application. Although incubation also reduced the concentration of Zn extracted from soils by DTPA, the changes in DTPA-extractable Zn were not always consistent with the yield of subterranean clover.

Soil tests for Zn are one way of estimating its availability to plants and should help to predict the likelihood of a response to applied Zn before a crop is grown. Extractants with a wide soil : solution ratio and a high ionic strength, such as $0 \cdot 1$ M HCl (Trierweiler and Lindsay 1969), estimate quantity, while chelating agents such as DTPA are considered to measure both quantity and intensity (Lindsay and Norvell 1978). Extractants of neutral salts with low ionic strength such as $CaCl_2$ will estimate intensity. Changes with time in the amount of Zn extracted must reflect changes in the availability of Zn for plant uptake.

The objectives of our study were to measure the effect of incubation of applied Zn on the availability of Zn to navy beans and on Zn extracted by HCl, EDTA, DTPA and dilute $CaCl_2$.

Table 1. Some properties of the soils used (air-dry basis, 0–10 cm)

Soil	Loca-tion[A]	Classif-ication[B]	pH[C]	OC[D] (%)	Fe (%)	Al (%)	Sand (%)	Silt (%)	Clay[E] (%)
1	WH (W.A.)	Uc 5·22	6·2	0·5	0·02	0·02	92	1	8
2	Ta (W.A.)	Dy 3·81	6·5	3·0	0·05	0·43	79	6	15
3	M (W.A.)	Dr 2·33	6·9	1·6	0·03	0·06	72	7	21
4	T (Qld)	Gn 3·14	5·6	1·7	0·32	0·62	23	16	61

[A] WH, Wongan Hills; Ta, Talbot; M, Merredin; T, Tolga.
[B] Northcote (1974).
[C] 1 : 5 H_2O.
[D] Organic carbon.
[E] Gee and Bauder (1986).

Materials and Methods

Experiment 1. Effects of Incubation on Plant Growth

The experiment was a complete factorial of six rates of Zn application and two times of Zn application with three replications. Navy beans (*Phaseolus vulgaris* cv. Gallaroy) were grown in a gravelly sandy loam from Talbot (Table 1) and plants were harvested 22 and 33 days after sowing (d.a.s.).

Rates of Zn applied in solution as $ZnSO_4.7H_2O$ were 0, 0·17, 0·32, 0·50, 0·75 and $1 \cdot 0 \mu g g^{-1}$ soil. The times of application were Zn applied either before (incubated Zn) or after (recently applied Zn) a 15 day incubation at $40 \pm 1°C$. Each pot was lined with a polyethylene bag and contained 2490 g of oven-dry soil (0–10 cm, <4 mm). Basal nutrients were applied in solution to the soil surface of all pots (mg pot^{-1}): NH_4NO_3, 214; KH_2PO_4, 1757; K_2SO_4 328; $CaCl_2$, 441; $MgSO_4.7H_2O$, 64; $MnSO_4.H_2O$, 53; $CuSO_4.5H_2O$, 14; H_3BO_3, 1; $CoSO_4.7H_2O$, 0·86; $Na_2MoO_4.2H_2O$, 0·8. Analytical grade reagents were used and macronutrient solutions were purified with dithizone/chloroform (Hewitt 1952). After addition of the nutrient solutions, the soil surface was allowed to dry and the soil was emptied into a large plastic container and mixed, in order of increasing Zn application, before repotting. Water was added to 75% of field capacity [field capacity was 25% gravimetric moisture determined by the column method (Veihmeyer and Hendricksen 1950)] and the pots were covered with a plastic bag, sealed and stored in a constant temperature room at $40 \pm 1°C$ for 15 days. At the end of the incubation, the soil from each pot was emptied onto polyethylene sheeting and dried in a glasshouse for 4 days before repotting into the original liners. The recently applied Zn treatments and a second application of P to make a total of 800 mg P pot^{-1} were added to the soil surface on the day before planting. This rate of P application was shown in a preliminary pot experiment to be adequate but not toxic for the growth of navy beans on this soil. All pots were mixed as previously and repotted.

Six seeds of *Phaseolus vulgaris* cv. Gallaroy (Zn content of 6 µg seed^{-1}) were planted in April 1987 and the pots were watered to 60% of field capacity with double deionized water. The pots were placed in root cooling tanks at 25°C and re-randomized weekly on a replicate basis. Pots were watered as required to 60% of field capacity until 6 d.a.s. when the plants were thinned to three uniform plants per pot. For the remainder of the experiment, the pots were watered to field capacity by weight. Additional N (214 mg pot^{-1} of NH$_4$NO$_3$) was added to the pots before watering to weight on 9, 17, 23 and 29 d.a.s.

The first harvest was made 22 d.a.s. when plants were cut 2 cm above the soil level and weighed. The stems were washed with deionized water to remove adhering soil and the plants were sectioned into young leaf, youngest open leaf, youngest fully expanded leaf and remainder of shoots. The parts were wrapped in tissue, placed in envelopes and oven-dried at 60°C for 48 h. Plants were harvested in a similar manner 33 d.a.s. The plant samples were digested in HNO$_3$/HClO$_4$ (Johnson and Ulrich 1959) and Zn was measured by flame atomic absorption (Allan 1961).

After seedling emergence (3 d.a.s.), eight soil cores of 1 cm diameter were taken from each pot with a stainless steel tube, air-dried and mixed. The soil samples were analysed for DTPA-extractable Zn (Lindsay and Norvell 1978) and 0·002 M CaCl$_2$-extractable Zn.

Experiment 2.   Effect of Incubation on the Chemical Extraction of Zn from Soils

Four soils (Table 1) were incubated with Zn for 0, 0·25, 0·5, 1, 2, 4 and 8 days at 40±1°C and analysed for 0·1 M HCl-, EDTA-, DTPA-extractable Zn, and 0·01 and 0·002 M CaCl$_2$-extractable Zn.

Air-dry soil, equivalent to 120 g of oven-dry soil, was weighed into separate polyethylene bags for each incubation treatment on day 0 and between 13 and 35 ml of Zn solution was added to each bag to apply 2·5 µg Zn g^{-1} of oven-dry soil and to wet the soil to field capacity. Field capacities were 11, 25, 21 and 37% for soils 1, 2, 3 and 4, respectively. The bags were heat-sealed, mixed thoroughly and placed in an oven at 40°C for 0, 0·25, 0·5, 1, 2, 4 or 8 days. At the end of each incubation period, the bags were removed from the oven and the soil was weighed into polycarbonate vials. Sufficient soil was weighed into each vial so that the oven-dry weight of soil was equivalent to the amount required for the extraction by one of the methods described below. The vials were then frozen until the end of the experiment, when they were thawed to room temperature and extracted in one batch for each soil extraction method. There were three replicates of each treatment.

The methods used for soil analysis were (i) 0·1 M HCl (Tiller *et al.* 1972; 30 min shake at 1 : 20 soil/solution ratio); (ii) 0·005 M EDTA and 0·01 M Ca(NO$_3$)$_2$ (Fujii and Corey 1986); (iii) 0·005 M DTPA (Lindsay and Norvell 1978); (iv) 0·01 M CaCl$_2$; (v) 0·002 M CaCl$_2$. Samples for methods (iv) and (v) were shaken for 16 h as a soil/solution ratio of 1 : 5. The CaCl$_2$ extracting solutions were prepared from a stock solution that had been purified with dithizone/chloroform. After centrifugation, all extracts were filtered (<0·45 µm; CaCl$_2$ extracts were acidified to 0·1 M with HCl) and analysed by flame atomic absorption. Flameless atomic absorption was used when Zn concentrations were less than 0·1 µg ml^{-1} Zn.

Organic carbon was determined by the method of Walkley and Black (1934) with a colorimetric modification (Sims and Haby 1971). The Fe and Al were extracted with acid ammonium oxalate at a soil : solution ratio of 1 : 40 (McKeague and Day 1966) and analysed by atomic absorption (Searle and Daly 1977). Particle-size analysis was determined by the pipette method after shaking soil in a solution of 2·5% sodium hexametaphosphate in 0·1 M NaOH (Gee and Bauder 1986).

Results

Experiment 1.   Effects of Incubation on Plant Growth

(1) Plant symptoms

Symptoms of Zn deficiency began to appear 15 d.a.s., as faint interveinal chlorosis of the trifoliate leaves of most plants from the nil Zn treatment and some plants from the 0·17 µg Zn g^{-1} soil treatments, and became more

severe with time. Primary leaves of the same plants developed a red-brown colouration of the veins that progressed to water-soaked areas and necrosis. At the second harvest (33 d.a.s.), plants from the nil Zn treatment were stunted and primary leaves had severe red veination with necrotic patches while the first trifoliate leaves were small, droopy and had curled margins. These symptoms are similar to those reported previously (Viets *et al.* 1954; Boawn *et al.* 1969; Wade 1985), but red veination, the most obvious symptom on the primary leaves, has not been reported, although Viets *et al.* (1954) mention brown spots in the mesophyll.

Plants grown with incubated Zn had more severe symptoms than those grown with recently applied Zn, while symptoms for both times of application decreased in severity as the Zn rate increased. Plants grown with the highest rate of recently applied Zn (1 μg Zn g^{-1} soil) had no symptoms, whereas plants grown with the same amount of incubated Zn had necrotic areas on the first trifoliate leaves.

(2) *Growth and Zn uptake*

The application of Zn increased the dry weight of navy bean shoots at both harvests. At harvest 1 (22 d.a.s.), dry weight increased linearly with applied Zn and there were no consistent differences between the dry weight of plants grown with recently applied and incubated Zn (Table 2). Yield in the absence of applied Zn was 61% of the maximum yield obtained when 1 μg Zn g^{-1} soil was applied. At the second harvest, the dry weight of the recently applied Zn treatments was consistently higher than that of plants grown with the same amount of incubated Zn, except at the highest Zn rate (1 μg Zn g^{-1} soil) where yields were similar (Fig. 1). The concentration of Zn in the youngest open leaf indicated that maximum yield had been obtained with the highest rate of Zn addition (Armour *et al.*; unpublished data). Linear regressions with a common intercept were fitted to the linear part of the response curves (Fig. 1). The relative effectiveness for plant growth, calculated by dividing the slope of the regression for dry weight of shoots against Zn applied for incubated Zn by the slope for the regression for recently applied Zn (Barrow and Campbell 1972), was 0·68. Plants grown without added Zn were severely Zn-deficient and the dry weight increased by only 9% between the two harvests, compared with 256% for plants grown with the highest Zn rate.

The Zn uptake increased linearly with rate of Zn addition for both recently applied and incubated Zn applications at harvest 1 (Table 2; $Y = 17 \cdot 50 + BX, B = 33 \cdot 06$ and $23 \cdot 59$ respectively, $r^2 = 0 \cdot 86$) and harvest 2 (Fig. 2). Relative effectiveness for Zn uptake (calculated in a similar manner to that for plant growth) was approximately the same for harvests 1 and 2 (0·71 and 0·80, respectively). The ratio of DTPA-Zn extracted from incubated Zn treatments to that extracted from recently applied Zn was 0·76–0·86 and was not related to rate of Zn application (Table 2).

At each harvest, there was also a consistent relationship between dry weight and DTPA-Zn that was independent of the time of Zn application. The relationship was linear at harvest 1 ($r^2 = 0 \cdot 83$), but better explained by a Mitscherlich relationship at harvest 2 (Fig. 3). Concentrations of Zn in 0·002 M CaCl$_2$ extracts were close to the concentrations in the blank solution and could not be measured.

Table 2. **Dry weight and Zn uptake of navy bean shoots and DTPA-Zn for recently applied and incubated Zn applications for harvest 1 (22 d.a.s., experiment 1)**

| Zn rate ($\mu g\ g^{-1}$)	Dry weight of shoots ($g\ pot^{-1}$)		Zn uptake ($\mu g\ pot^{-1}$)		DTPA-Zn ($\mu g\ g^{-1}$)		Relative concentration of DTPA-Zn[B]
	Recent[A] Zn	Incubated Zn	Recent Zn	Incubated Zn	Recent Zn	Incubated Zn	
0	2·04	2·04	18·3	18·3	0·07	0·07	
0·17	2·23	2·08	24·5	19·0	0·10	0·08	0·81
0·32	2·10	2·50	23·2	25·7	0·11	0·09	0·76
0·50	2·73	2·75	42·3	32·3	0·14	0·11	0·81
0·75	2·97	2·36	37·8	31·1	0·18	0·14	0·80
1·00	3·44	3·23	51·3	43·4	0·23	0·21	0·86

[A] Recently applied Zn.
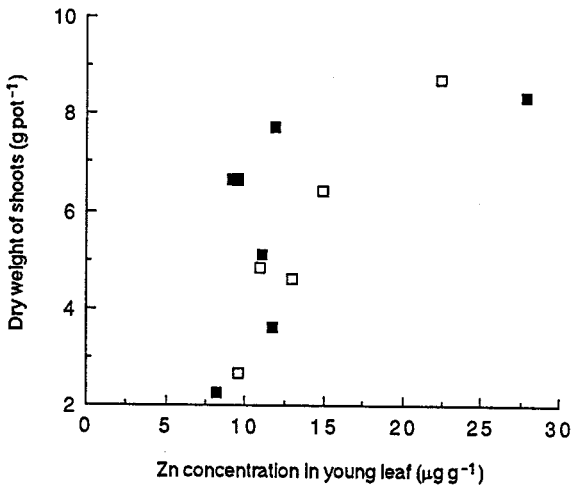[B] Incubated DTPA-Zn/Recently applied DTPA-Zn.
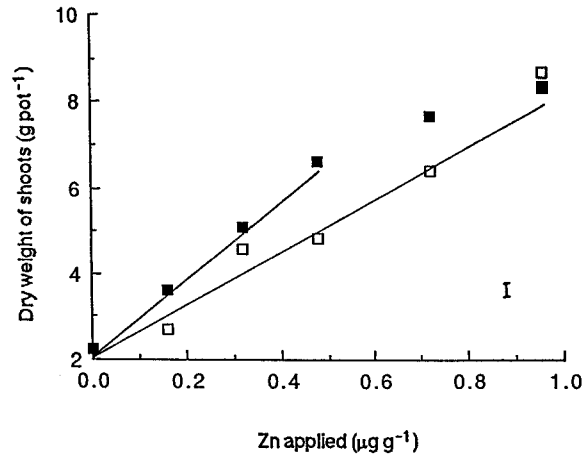
Fig. 1. Effect of recently applied (■) and incubated (□) Zn applications on the dry weight of navy bean shoots at harvest 2 (experiment 1) and fitted linear regressions of $Y = A+BX$, where $A = 2\cdot04$ and $r^2 = 0\cdot97$, $B = 9\cdot33$ for recently applied Zn and $6\cdot34$ for incubated Zn. The vertical bar denotes the standard error.
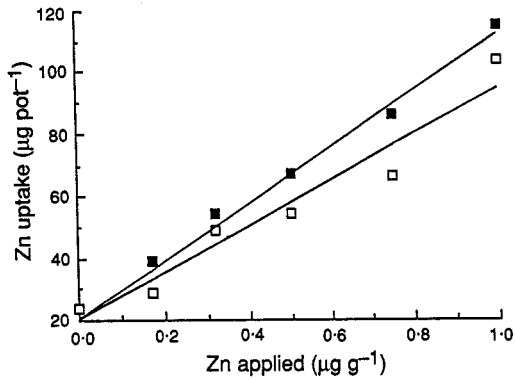
Fig. 2. Effect of recently applied (■) and incubated (□) Zn applications on Zn uptake by navy bean shoots at harvest 2 and fitted linear regressions of $Y = A+BX$, where $A = 20 \cdot 55$ and $r^2 = 0 \cdot 97$, $B = 93 \cdot 23$ for recently applied Zn and $74 \cdot 73$ for incubated Zn.
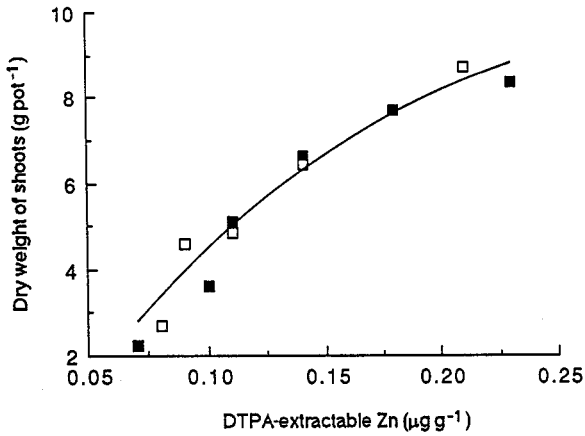
Fig. 3. Relationship between dry weight of navy bean shoots at harvest 2 and DTPA-extractable Zn for recently applied (■) and incubated (□) Zn applications, and fitted Mitscherlich curve, $Y = 11 \cdot 82 - 14 \cdot 43e^{-6.75x}$, $r^2 = 0 \cdot 86$ (experiment 1).

Fig. 4. Relationship between dry weight of navy bean shoots at harvest 2 and Zn concentrations in young leaves for recently applied (■) and incubated (□) Zn applications (experiment 1).

Table 3. Zinc concentration on an oven-dry basis in untreated soil and after the addition of $2 \cdot 5 \, \mu g$ Zn g^{-1} soil measured by five chemical extractants (experiment 2)

Soil No.	HCl ($\mu g \, g^{-1}$)		EDTA ($\mu g \, g^{-1}$)		DTPA ($\mu g \, g^{-1}$)		$0 \cdot 01$ M CaCl$_2$ ($\mu g \, kg^{-1}$)		$0 \cdot 002$ M CaCl$_2$ ($\mu g \, kg^{-1}$)	
	Untreated	Fert.[A]	Untreated	Fert.	Untreated	Fert.	Untreated	Fert.	Untreated	Fert.
1	0·07	2·25	0·04	2·21	0·03	1·87	6·5	1580 (1320–1795)[B]	2·0	1176 (950–1420)
2	0·27	2·45	0·13	2·06	0·07	1·20	<2	11·0 (8·5–15·0)	<2	8·8 (7·9–11·1)
3	0·83	2·80	0·61	2·60	0·39	1·78	<2	<2	—	—
4	1·95	3·13	0·90	1·36	0·73	1·37	3·0	24·5 (19·5–30·5)	4·3	8·3 (7·5–9·7)
s.e.	0·02	0·24	0·02	0·13	0·02	0·12	0·33	80	0·35	79

[A] Zn concentration at the first incubation time of 0·03 days at 40°C.
[B] Range of replicate data.

Table 4. Coefficients and r^2 for the regression of the relative concentration of Zn extracted by five methods with time of incubation for the model $Y = Ct^B$ (experiment 2)

Soil	HCl			EDTA			DTPA			$0 \cdot 01$ M CaCl$_2$			$0 \cdot 002$ M CaCl$_2$		
	B	C	r^2	B	C	r^2	B	C	r^2	B	C	r^2	B	C	r^2
1	−0·060	0·733	0·77*	−0·066	0·696	0·96**	−0·124	0·473	0·98**	−0·117	0·413	0·95**	−0·144	0·274	0·94*
2	−0·181	0·459	0·88**	−0·230	0·345	0·97**	−0·337	0·143	0·97**	−0·340	0·001	0·89**	−0·271	0·001	0·87*
3	−0·025	0·710	0·79**	−0·041	0·732	0·66*	−0·072	0·441	0·88**						
4	−0·327	0·177	0·90**	−0·546	0·047	0·70*	−0·713	0·029	0·76*	−0·695	0·001	0·80**			

* $P < 0 \cdot 05$; ** $P < 0 \cdot 01$.

Incubation did not appear to affect the relationship between dry weight and the Zn concentration in plant tissue at each harvest (Fig. 4; harvest 1 data not presented).

Experiment 2. Effect of Incubation on the Chemical Extraction of Zn from Soils

The addition of $2 \cdot 5 \, \mu g$ Zn g^{-1} soil increased the concentration of Zn extracted by each method at all times of incubation compared with concentrations of the untreated soil (Table 3). The largest increases in Zn concentration after the addition of Zn occurred in the $CaCl_2$ extracts and the smallest increases were generally in DTPA.
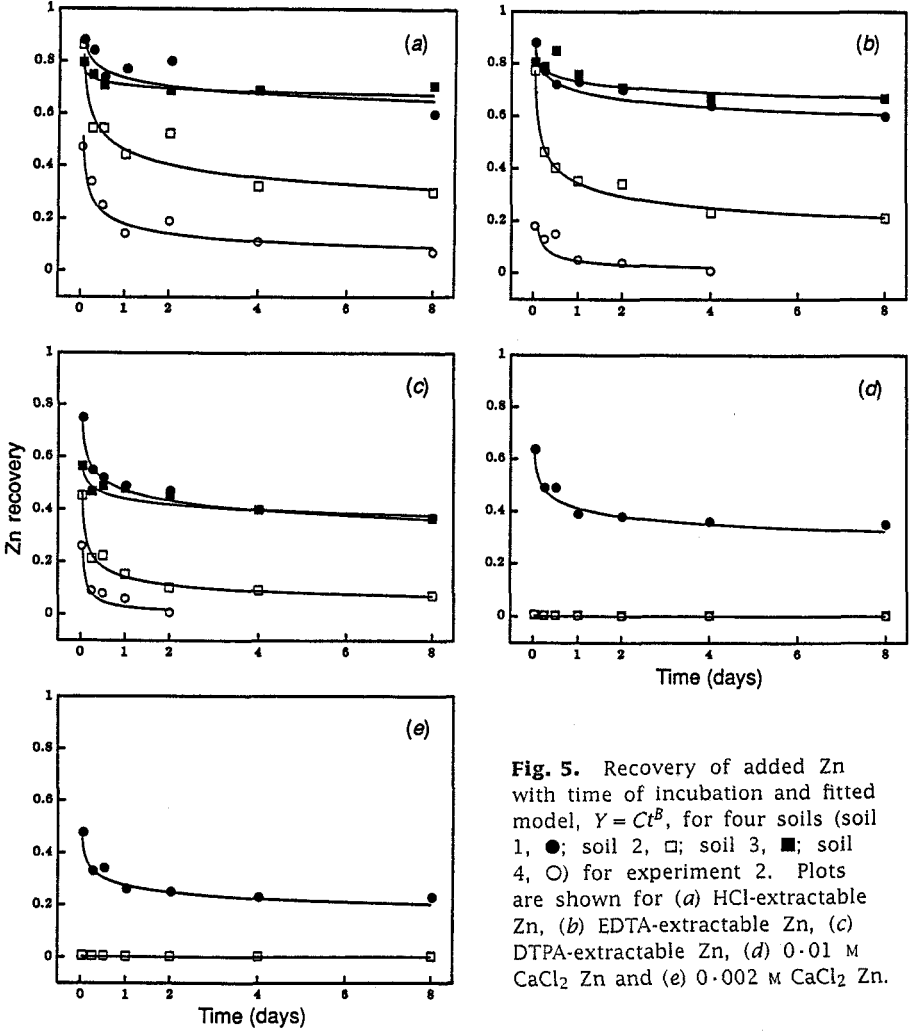
Fig. 5. Recovery of added Zn with time of incubation and fitted model, $Y = Ct^B$, for four soils (soil 1, ●; soil 2, □; soil 3, ■; soil 4, ○) for experiment 2. Plots are shown for (*a*) HCl-extractable Zn, (*b*) EDTA-extractable Zn, (*c*) DTPA-extractable Zn, (*d*) $0 \cdot 01$ M $CaCl_2$ Zn and (*e*) $0 \cdot 002$ M $CaCl_2$ Zn.

In all soils, the Zn concentration in each extract decreased exponentially with increasing time of incubation, indicating that none of the extractants are a measure of the total Zn concentration of the soil. The extent and rate of the decrease differed between soils and extractants. For each soil and extractant, a recovery of Zn was calculated by the formula, {[Zn] extracted −[Zn] native}/2·5,

where $2 \cdot 5\,\mu g\,Zn\,g^{-1}$ was the rate of Zn addition to the soil (Fig. 5). We assumed that incubation of the soil did not affect the extractability of native Zn. On average, between 50% and 77% of the added Zn was recovered by DTPA, EDTA and HCl extractants at the first incubation time. In contrast, the recovery by the $CaCl_2$ solutions was very low (<1%) in soils 2 and 4 but high in soil 1 (49–63%). Data from soil 3 were excluded because of the inability to obtain a clear extract with $0 \cdot 002\,M\,CaCl_2$ and erratic results for $0 \cdot 01\,M\,CaCl_2$.

The model, $Y = Ct^B$, was used to describe the relationship between Zn recovery and time of incubation. The time between the addition of Zn to the soil and the freezing of the samples was calculated by the Arrhenius equation (Castellan 1970) to be equivalent to $0 \cdot 03$ days at 40°C and this was used for the first incubation time in the model (Fig. 5). For soil 4, the concentration of Zn extracted at incubation times of 4 and 8 days for DTPA and at 8 days for EDTA was not different to the concentrations in virgin soil so the model was truncated at incubation times of 2 and 4 days for DTPA and EDTA, respectively, for this soil. The model generally provided a good fit to the data (Table 4) and most coefficients of determination were significant at $P < 0 \cdot 01$, but it was not fitted to data for $0 \cdot 002\,M\,CaCl_2$ for soil 4 because of the very low recovery at all incubation times. The coefficient, C, is a measure of the recovery of Zn after 1 day of incubation and the recovery increased with increasing C. Coefficient B is an estimate of the rate of decrease of Y; this rate increased as B decreased.

Of the HCl, EDTA and DTPA extractants, HCl had the highest C and B coefficients, DTPA had the lowest and EDTA was usually intermediate. For these extractants, C values were in the order of soil 4 ≪ soil 2 ≪ soil 3, soil 1. The B coefficients for all extractants were in the order soil 4 < soil 2 < soil 1 < soil 3, indicating that the greatest rate of decrease occurred in soil 4.

Another model, $Y = (1+Kt)^{-B}$ (Barrow 1980), was also fitted to the data. However, because this model requires the initial Zn concentration to be set at 1, the large differences between the soils and extractants in the recovery for this incubation time were not obvious.

Discussion

Incubation of applied Zn with the soil decreased both the availability of Zn to navy beans and the quantity of Zn extracted by the five soil extractants. The decrease in availability appeared to be caused by continuing reactions within the soil that converted some of the applied Zn into a form that is unavailable for plant uptake. The rate of the reaction as measured by experiment 2 was rapid initially, with a maximum of 87% of the added Zn recovered after the first incubation period of $0 \cdot 03$ days, and then continued more slowly for up to 8 days (Fig. 5).

There were different rates of decline of Zn recovery in the four soils and between the different extractants used in the laboratory experiment. Comparisons of the 'instant' recoveries of added Zn by HCl, EDTA and DTPA in the soils at the incubation time of $0 \cdot 03$ days show that the reaction of Zn with the soil was more rapid in soil 4 than in the other soils (Table 3). The reactions would have occurred during the period between the addition of $2 \cdot 5\,\mu g\,Zn\,g^{-1}$ soil and when the samples were frozen after weighing. The low recovery of Zn at all incubation times from soil 4 for all extractants may

be due to the high clay, silt, Al and Fe content of the soil. The recoveries for soil 2 had the next greatest rate of decrease and the difference in B and C coefficients for this soil compared with soils 1 and 3 may be due to the high organic carbon content and the comparatively high extractable Al concentration. The relationship between the soil organic fraction and cations has two contrasting effects. On one hand, soluble organic compounds may complex Zn and increase its concentration in the soil solution and its transport to roots, while insoluble organic compounds may act as a sink for these ions (Stevenson 1982; Chairidchai and Ritchie 1989).

Variation in the rate of decline of the recovery of Zn (i.e. the B coefficient) could reflect the number of sites available for adsorption. However, there were no consistent relationship between B and soil properties that is considered to be related to the ability of soil to adsorb nutrients. The lack of correlation could be due to the small sample size of four soils. Brennan and Gartrell (1986) were unable to relate different rates of decline in the availability of Zn to sub-clover with differences in pH, CEC, organic carbon, clay, Zn or free sesquioxides in 53 Western Australian, Queensland, South Australian and Victorian soils.

The extent of decline of the recovery of Zn was greatest for the intensity measures of available Zn (0·002 and 0·01 M CaCl$_2$) and smallest in the quantity measure that extracted the most Zn (0·1 M HCl).

The common relationships in experiment 1, for dry weight of shoots as a function of DTPA-Zn and for dry weight versus plant Zn concentrations for the recently applied and incubated Zn treatments (Fig. 3 and Fig. 4), are an important result. These relationships, and the similar dry weights at harvest 2 for both times of Zn application at close to maximum growth (1 μg Zn g^{-1} soil), show that the effects of incubating the soil in experiment 1 on plant growth were confined to reducing the availability of the added Zn.

The lack of effect of incubation on dry weight of shoots for harvest 1 may have been due to variability in the early stages of plant growth (reflecting the influence of seed size and vigor) or to a lower demand for Zn compared with harvest 2. The Zn uptake was clearly reduced by incubation for harvest 1. In other studies with copper, Brennan et al. (1980) also reported that similar estimates of relative effectiveness of incubated copper were obtained for dry matter production and copper uptake although, as in this work, the relative effectiveness for plant growth was slightly lower than that for copper uptake.

For a particular soil, if the major factor affecting the availability of recently applied Zn is reactions that continue to convert the Zn into an unavailable form, then a useful characteristic of the soil test (apart from a uniform relationship with plant growth and nutrient uptake) would be a large decrease with time. However, a good correlation between the two parameters is not necessarily sufficient because the shape of the relationship can affect the accuracy of the prediction. Large changes in the values of a potential soil test are a good characteristic because the variation of plant growth with the soil test will tend to be gradual and therefore will make more accurate predictions of availability than a relationship where plant growth varies enormously with a small change in the value of the soil test. The results from experiment 1 indicated that at lower rates of Zn addition (<1 μg g^{-1} soil), even though the change in Zn

extracted by $0 \cdot 002$ M $CaCl_2$ may have been large, the absolute values are very small and close to or below the detection limit for Zn analyses and therefore prone to large errors. The Zn extracted by the quantity estimates of available Zn were all within the detection range of the analytical method.

Incubated Zn in experiment 1 appeared to be more available to plants than would be anticipated from the changes of DTPA-Zn with time in experiment 2. In experiment 1, Zn extracted by DTPA after 15 days incubation at 40°C was 80% of Zn extracted by DTPA from the soils containing recently applied Zn. In comparison, DTPA-Zn in the same soil in experiment 2 decreased rapidly so that Zn extracted after 8 days was only 15% of that at the initial incubation period of $0 \cdot 03$ days. However, the comparison in experiment 1 was made between Zn extracted after 15 days of incubation and the equivalent of $0 \cdot 75$ days incubation at 40°C (as this was the period between the addition of the recently applied Zn and collection of soil samples). The results of experiment 2 (Fig. 5c) showed that the decreases in DTPA-Zn in the first $0 \cdot 75$ days of incubation were substantial and far greater than the decrease in DTPA-Zn at times after $0 \cdot 75$ days. In experiment 1, most of the change in extractability of Zn would have occurred before the plants were grown (and the soils were analysed) and, hence, incubated Zn was only slightly less available than recently applied Zn. There were also different rates of Zn addition in the two experiments. Additions of Zn were a maximum of $1 \cdot 3 \, \mu g \, Zn \, g^{-1}$ soil in experiment 1 (adjusted from $1 \cdot 0 \, \mu g \, g^{-1}$ to allow for incubation at 75% of field capacity compared with 100% in experiment 2) and $2 \cdot 5 \, \mu g \, g^{-1}$ in experiment 2. Barrow (1986) has shown, during the reaction of Zn with soil, that the amount of Zn retained in the soil increases with increasing solution Zn concentration.

In conclusion, the availability of Zn to navy beans and the concentration of Zn measured in a range of chemical extractants both decreased when Zn was incubated with the soil for a relatively short period of time. The rate and extent of the decrease in the extractant varied widely with soil type.

Acknowledgments

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