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# Uptake of cadmium and zinc by ryegrass at high solution culture levels

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## Summary

New top growth of ryegrass exposed to various solution culture levels of cadmium and zinc absorbed the elements without detectable interaction between the two. When after repeated clippings the contents had risen to 100 mg cadmium/kg DM or 700-1000 mg zinc/kg DM the new growth was injured. Interaction of zinc in cadmium toleration was suggested by the response of growth but could not be established with certainty.

## Introduction

In previous work ryegrass in Cd-containing solution culture was clipped at 12-day intervals. After each defoliation more Cd moved to the new top growth and its concentration increased in successive clippings eventually to 100 mg Cd/kg DM with consequent injury. At very high ambient levels the uptake rose quickly to still higher and lethal concentrations in the new growth with a sharp drop in yield of a few heavily injured clippings and subsequent death of the plants (Dijkshoorn et al., 1974).

The present work concerns Cd-Zn combinations. The highest Cd dose was that intended to produce toxic contents in later clippings. The highest dose of Zn was equimolecular to that of Cd.

In view of their chemical similarity some degree of interaction between Cd and Zn was expected, and the treatments were planned to test its occurrence.

## Material and methods

The technique was that of the previous communication. Cd was applied at rates of nil, 20, 40, 60, 80, 100 and 120 $\mu$mol Cd/litre, further denoted as Treatments a – g. To all nutrient solutions 0.8 $\mu$mol Zn/litre was added along with the trace elements needed for growth. In the Cd Series only Cd was varied at this low constant supply of Zn. In the Cd-for-Zn Series extra 120 $\mu$mol Zn/litre was added at nil Cd and, while Cd was raised, Zn was diminished by amounts equivalent to the added Cd with Cd + Zn constant at 120 $\mu$mol/litre. Cd and extra Zn were added as the sulphates in standard solutions containing 2 mol $Na_2H_2EDTA$/mol Cd or Zn and 3 mol KOH/mol Cd or Zn for neutralization.

Freshly defoliated sets of plants, previously established in Hoagland nutrient solutions, were transferred to those containing Cd on 29 August. The grass was clipped five times at successive intervals of 12, 15, 17, 21 and 25 days. Growth durations were made longer to compensate for the decrease in input of light in the autumn greenhouse climate. This and extra artificial lightening could not prevent a drop in yield and after 5 clippings the experiment was suspended.

## Results and discussion

Uptakes were calculated as kg DM in the new top growth multiplied by the measured concentration in mg Cd or Zn/kg DM, and cumulative uptakes and yields by adding the uptakes and dry weights of previous clippings to those of the succeeding one. These total outputs are shown in Fig. 1 for the Cd-for-Zn series of treatments. To cover the wide range of metal outputs, equal logarithmic scales were used for Cd and Zn as ordinates and dry weights as abscissas. In this type of graph proportionality and consequent constancy of mg/kg DM are indicated by straight 45° lines.

To obtain convenient plots with the units mg Cd or Zn and kg DM in whole numbers, all quantities per pot have been multiplied by 100. From the intercept

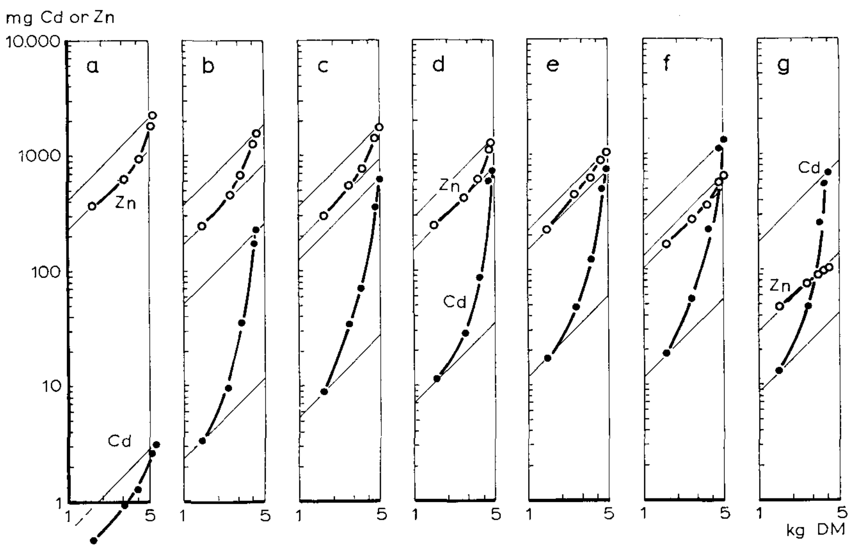


Fig. 1. Cumulative uptakes of mg Cd (solid dots), and mg Zn (open dots) per 100 pots, plotted against the cumulative yields of DM at the Treatments a – g with the various Zn–Cd combinations.

of a 45° line through a point with the ordinate raised at 1 kg DM, the corresponding concentration in the conventional unit mg/kg DM can be read off.

Height and width of the curves are a measure of the progressive relative increments from Cut 1 to the combined Cuts 1 – 5. Repeated clippings raised the yield from 1.5 to 5 kg DM/100 pots or 3.5-fold in the 90-day test period. The DM range was narrower than the 6-fold increase for the first five cuts of the previous experiment obtained within 60 days. But the 60-fold range in Cd output was wider and the present experiment produced more upright curves with steeper slopes: Cd concentrated with the number of fall clippings of longer growth duration more steeply than occurred in the summer climate with rapid growth maintained in full light. Higher concentrations of plant constituents in autumn, when the growth rate falls, is a common observation.

The intercepts with the ordinate at 1 kg DM of the 45° lines bracketing the curves show that treatments c – f produced first cuts containing 5 – 10 mg Cd/kg DM and, throughout the test period, combined Cuts 1 – 5 with 100 – 250 mg Cd/kg DM. The treatments a–f were equally productive and the new growth remained healthy in appearance. Only at the Highest Cd–Lowest Zn treatment (g) the later new growth looked injured with a drop in total output from 1300 to 700 mg Cd and from 5 to 4 kg DM/100 pots. The concentration of Cd in the combined five clippings dropped from 260 at treatment f to 175 mg Cd/kg DM at treatment g. Change in leaf colour, smaller new growth, and less mg Cd/kg DM were the effects of prolonged exposure to injurious amounts of Cd in the tissues already known from the previous experiment.

Uptake of Zn was highest at the Lowest Cd–Highest Zn treatment (a), and declined from treatment a to treatment g when more of the added Zn had been replaced by Cd in the medium. The upward-trending curves with slopes steeper than 45° show that each succeeding cut contained more Cd and Zn relative to DM than the previous one: concentrations in the new growth increased with the number of previous clippings. Only at the Highest Cd–Lowest Zn treatment (g) the dots for Zn lie along a 45° line as for proportionate increments at a nearly constant low level of 30 mg Zn/kg DM.

In the Cd series with Cd as the only variable at low constant Zn, injured and smaller new growth at higher Cd ranged from treatment d to treatment g, instead of being restricted to the heaviest Cd treatment (g) as in the Cd-for-Zn series. From this it seemed that extra Zn enabled the plants to withstand a higher level of Cd than they could ordinarily tolerate.

It may be well at this stage to continue with the original measurements of concentrations in the individual clippings. The frequently renewed media underwent only minor depletion of Cd and Zn which means that differences in yield cannot have led to differences in the degree of exhaustion as an extra source of differences in their concentrations in the grass.

To accentuate relative changes over the wide range of concentrations in the new growth of the individual Cuts 1 – 5 these have been plotted as ordinates along a logarithmic scale in Fig. 2 and 3. The same scale was used to plot the yields in dekagrammes DM/pot. The abscissas denote the external concentrations of Cd

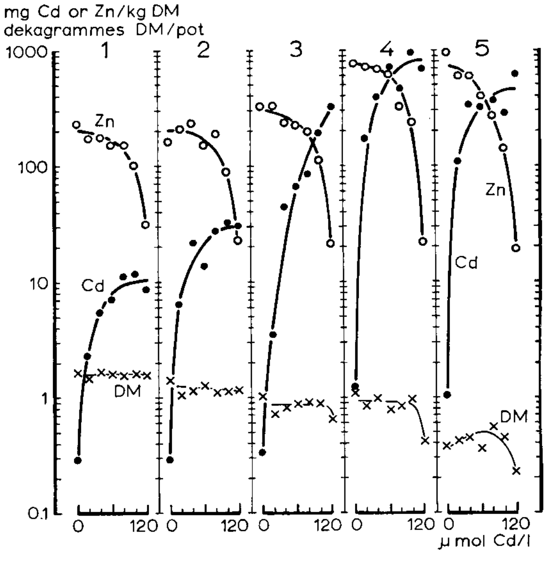


Fig. 2. Concentrations of Cd and Zn in mg/kg DM in the successive Cuts 1 – 5 produced by the Cd–for–Zn treatments. Abscissas: external concentrations of Cd. Yields (crosses) in dekagrammes DM/pot.

with or without partnered Zn.

Fig. 2 concerns the Cd-for-Zn series. After the decrease in Zn with the increase in supply of Cd, the descending curves for Zn are mirrored by ascending curves for Cd.

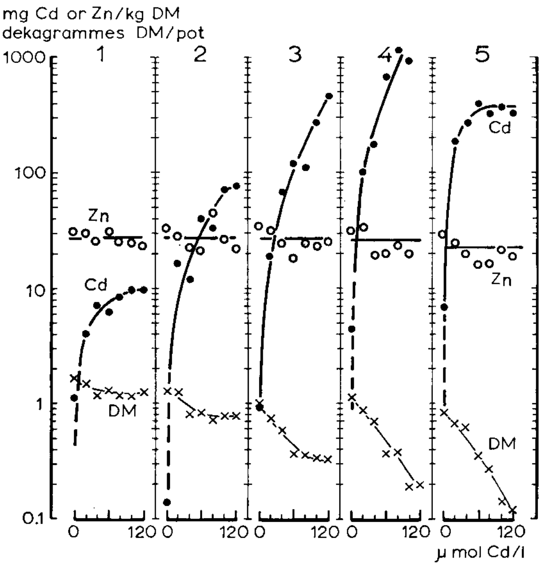


Fig. 3. Same as Fig. 2, but for the treatments with Cd as the only variable at low constant Zn.

Fig. 3 is for the Cd series with increasing Cd as the only variable, in which Zn in the new growth remained low and constant at about 20–30 mg Zn/kg DM, and Cd increased as in Fig. 2.

Cd rose steeply in the tops with the first few additions of Cd and tended towards a plateau at high Cd which was least in Cut 1 (10 mg Cd/kg DM) and highest in Cut 4 (about 800 mg Cd/kg DM). The maximum was not a constant character of the new growth but increased with the number of previous clippings, and presumably followed a progressive increase in the amount of Cd stored by the lower portions of the plant that remained after defoliation.

A true maximum was attained at Cut 4 at which injurious amounts of Cd were spread out over the whole plant with partial obstruction of the transfer system to yield lower maximum concentrations of Cd in the subsequent new top growth of Cut 5.

As Cd is not essential for growth, there will be no lowest possible limit in mg Cd/kg DM. Zn, a nutrient essential for growth, has a minimum of the order of 10 mg Zn/kg DM which permits no further dilution by DM gain, and would stop the growth upon removal of Zn from the supply. Its upper limit is set by toxicity and must be of the order of 700–1000 mg Zn/kg DM, because at this level of tissue Zn the new growth of Cut 5 at Nil Cd–Highest Zn (Fig. 2) was small and injured whereas at Nil Cd–Low Zn (Fig. 3) it was higher yielding and healthy. There would remain a 100-fold range of possible Zn contents from the minimum metabolic demand up to toleration of excess.

Toxicity produced small injured growth of Cut 5 at all the Cd-Zn combinations of Fig. 2 as at low Zn, high Cd and at low Cd, high Zn had become toxic for growth. In the Cd series with low Zn, the plants were injured only by high Cd and the yield of Cut 5 dropped from an initially higher value more steeply with the increase in Cd in Fig. 3 than it did among the lower yielding Co-for-Zn treatments of Fig. 2 which merely changed from toxic Zn to toxic Cd.

A survey of the graphs reveals that toxic levels exceeding 100 mg Cd/kg DM were attained from the third cutting date onwards. In Fig. 3 there remains no doubt that the yield of DM dropped under the influence of higher Cd: growth of Cuts 3, 4 and 5 was smaller and visibly injured in the range of the heavier treatments. But in Fig. 2 with the Cd-Zn combinations only the Highest Cd–Lowest Zn treatment was toxic. From the first substitution of one-sixth of the maximum Cd dose by Zn the Zn content increased from 20 to 100 mg Zn/kg DM and higher, and these plants with extra Zn remained healthy and grew well until high Zn became toxic in the later new growth of Cut 5.

The identical Highest Cd treatments of Fig. 2 and 3 differed in yield of DM. A contributory source to this dispersion was an incidental decline of the original stand density among the sets of plants used in Fig. 3 as reflected by a 20 % yield reduction at pre-treatment harvest and in Cut 1 with still non-toxic levels of Cd. But the effect of this planting error was small compared with that of treatment.

The more important source of dispersion was an unexplained quicker accumulation of Cd in Fig. 3 even at the identical Highest Cd–Lowest Zn treatments of Fig. 2 and 3 as the evidence that it was independent of extra Zn. At the midpoint treatment, with 60 $\mu$mol Cd/litre and with or without 60 $\mu$mol Zn/litre, Cuts 1, 2, 3 and 4 contained 7, 22, 65 and 500 mg Cd/kg DM respectively, with high tissue Zn in Fig. 2 and 7, 30, 100 and 500 mg Cd/kg DM, respectively, with low Zn in Fig. 3. At high Zn the repeated new growth remained healthy and productive, but at low Zn all the new growth containing 100 mg Cd/kg DM or more was hit by Cd toxicity with a 50 % reduction in yield.

Cut 4 alone would suggest that the 500 mg Cd/kg DM was toxic at low Zn in Fig. 3, but rendered non-toxic by the extra 600 mg Zn/kg DM contained by the new growth of Fig. 2. But, perhaps by chance, the previous new growth of Cut 3 contained 100 mg Cd/kg DM at low Zn, but only 65 mg Cd/kg DM at high Zn. As tolerance involves time of exposure, not high Zn, but the lower Cd content of the preceeding Cut 3 may have contributed to the greater tolerance to 500 mg Cd/kg DM measured at the fourth cutting date because it came from a lower, less toxic level during the first new growth of Cut 4 than was the case in the plants at low Zn and Cut 3 already at 100 mg Cd/kg DM.

The data of Cut 5 are not forthcoming in the decision because at test conclusion the onset of Zn toxicity at Low Cd–High Zn (Fig. 2), and of obstruction in the uptake mechanism of Cd at High Cd–Low Zn (Fig. 2 and 3), created a new situation which was the result rather than a continuation of the previous trend.

Largely on account of this difference in rate of Cd accumulation between the high and low Zn groups we are unable to establish whether high Zn protected the plants against toxicity of Cd, as was anticipated from the very conspicuous differences in response to high Cd levels between the plants high and low in tissue Zn.

In Fig. 2 the curves for Cd and Zn are both concave with respect to the horizontal axis of external concentrations which indicates that uptake and transport of Cd and Zn to the tops were of a non-competitive nature. The form of the Cd curves of Fig. 2 and 3 are similar. The combined evidence shows that Cd and Zn were delivered to the new top growth in a way completely independent of one another.

## Reference

Dijkshoorn, W., J. E. M. Lampe & A. R. Kowsoleea, 1974. Tolerance of ryegrass to cadmium accumulation. *Neth. J. agric. Sci.* 22: 66-71.