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Phytotoxicity of zinc, nickel, cadmium, lead, copper and chromium in three pasture plant species supplied with graduated amounts from the soil

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Summary

Narrow-leaved plantain, white clover and perennial ryegrass were grown on an acid sandy soil with added levels of Zn, Ni, Cd, Pb, Cu and Cr, as the single test elements.

The metals were classified for inhibitive metal concentrations in the shoots of the plants and in the soil.

Ni, Cd and Cu were found to cover a fivefold range with one exception: Cd in plantain was inhibitive at a tenfold higher tissue concentration than in clover and grass.

Zn was more efficiently absorbed, but tolerated at a higher soil concentration because it was much less toxic to the tissue. Cr was tolerated at low shoot concentrations but at high concentrations in the soil because its uptake by the shoots was disproportionally smaller.

Differences between plant species in response to the soil test levels were illustrated in terms of relative uptakes and shoot concentrations for toxicity.

Introduction

Possible sources of heavy metals in pastures are depositions of sewage sludge and dietary Cu in excreta from pigs.

Acute phytotoxicity of a sewage sludge to grass has been demonstrated by de Haan (1975a, b). On sludge alone the growth was only 4 % of that obtained on soil and soil-sludge mixtures. Toxicity was due to high Ni in the sludge (930 mg Ni/litre) and in the herbage (190 mg Ni/kg DM). Another sludge with 190 mg Ni/litre produced normal-yielding grass with 50 mg Ni/kg DM.

Removal by plant uptake from the soil is slow. Kelling et al. (1977) reported that four successive crops had removed only 2.6, 0.4, 0.3, 0.3 and 0.05 % of Zn, Cd, Ni, Cu and Cr, respectively, added with 3.75 tonnes of sludge per ha. Percentual recovery of the added Zn was fifty times greater than that of the added Cr.

Another measure of the relative uptake is the plant: soil concentration ratio of the metal. For cabbage grown on metal amended culture solutions Hara & Sonoda (1979) found ratios decreasing in the order Zn, Ni, Cd, Cu and Cr which parallels Kelling's series of decreasing uptakes from the soil.

In comparative studies on tolerance to soil metals the relative uptakes are important. Miles & Parker (1979) showed that among four plant species soil Cd was most toxic to the one absorbing the greatest amount of the metal from the soil. This suggests that in pasture vegetations the botanical composition may influence the metal content of the herbage. In turn, the type and degree of contamination may change the proportion at which the plant species will contribute to the new growth.

The metals are taken up through the roots which are not normally grazed. Entry into the food chain depends on translocation of the absorbed metals from the roots to the edible portions above the soil.

Cary et al. (1977) screened various plant species for the increase in shoot concentration after a short-term exposure of the roots to Cr in nutrient solutions. About 90 % of the absorbed Cr was retained in the roots, but an increased breakthrough to the tops was observed if more than 25 mg/litre were added to the solution.

Petterson (1976) found that Zn and Ni were readily translocated from the roots to the shoot, Cd and Pb less readily, and Cu and Cr to a small extent only. Hara & Sonoda (1979) found that in cabbage Zn, Ni and Cd moved to all vegetative parts, whereas Cr was strongly retained in the roots. Uptake and translocation of Cr III and Cr VI were equal as was also found by Huffman & Allaway (1973) and Petterson (1976). One-fourth of the absorbed Pb went to the shoots of ryegrass (Jones et al., 1973), and two-thirds to the shoots of oats (John & van Laerhoven, 1972).

Wallace et al. (1977a) showed that rice plants exposed to the metals in nutrient solution attained shoot: root concentration ratios of 1.2 for Zn, 0.1 for Cd, 0.03 for Pb and 0.004 for Cr.

Although the work quoted included other metals the extracted evidence has been confined to those to be considered in the present experiments. A likely generalization would be that the relative uptakes by the shoots of plants decline in the order: Zn, Ni, Cd, Pb, Cu, Cr mainly through a parallel decrease in the rate at which the metals are translocated from the roots to the shoots.

Literature for over many years has indicated that even the essential metals are toxic to plants in high concentration. Apart from the destructive action of excessive amounts in the soil associated with rapid wilting and death, higher tissue concentrations may produce healthy-looking but lower-yielding plants.

Hara & Sonoda (1979) found a reduction in the yield of cabbage when the outer leaves contained 1000, 100, 100, 50 and 10 mg/kg DM of Zn, Ni, Cd, Cu and Cr, respectively. The metals, arranged for decreasing toxicity, parallel the order of decreasing relative uptake and translocation to the shoots given earlier.

In ryegrass similar leaf concentrations for toxicity of Zn and Cd (Dijkshoorn et al., 1975) and Pb (Jones et al., 1973) were found. In lettuce, leaf contents of 140 mg Pb/kg DM reduced the yield (John & van Laerhoven, 1972). In various other plant species contents of 100 mg Ni, 70 mg Cd, and 30 mg Cu per kg DM were found to be inhibitive to the growth (Wallace et al., 1977a-e).

These values are no true tissue concentrations for toxicity. The sequence of metals parallels that of increasing retention in the roots, presumably associated with a basipetal shift of the site of first injury as the toxic level is attained earlier in the roots. It is likely that, for instance, the high toxicity of Cr suggested by leaf tests (10 mg Cr/kg DM) is due to its tenfold higher concentration in the roots.

However, for a given metal-plant combination the degree of partitioning of the metal between roots and shoots may be considered as sufficiently characteristic to warrant the use of the more practicable shoot analysis as an index to phytotoxic plant contents.

Research is being carried out in the Institute on the effect of metal-bearing sewage sludge amendments in pastures. In this setting the present pot experiments were undertaken to complement pasture research in a quest for levels of Zn, Ni, Cd, Pb, Cu and Cr in herbage and soil which are toxic to the growth of grass and herbs. Three species were used: *Plantago lanceolata* L., *Trifolium repens* L. and *Lolium perenne* L., to be further designated as plantain, clover and grass, respectively.

An unpublished review on toxic metal contents in plant and soil, written and kindly furnished by Dr Ir K. W. Smilde of the Institute for Soil Fertility at Haren, was consulted for the choice of appropriate levels in the soils.

Material and methods

Fresh single-salt solutions of the metals were prepared by dissolving appropriate quantities of the following salts: the sulphates of Zn, Ni, Cd, Cu and Cr III, and the acetate of Pb.

A sandy soil was used with 4.4 % organic matter, no calcium carbonate, and a pH of 4.7 measured in a 10:25 suspension in water.

For each treatment a 4 kg quantity of the soil was placed in a shallow container and blended with measured amounts of N, P, K and Mg in nutrient-salt solution as a constant basal dressing. Then a measured volume of one of the heavy-metal standard solutions was incorporated in the dressed soil.

For each metal series eight separate soil portions were prepared. Each included the uncontaminated soil, and the added metal levels were adjusted so as to obtain a stepwise twofold increase in the level of the singly supplied test element.

The basal dressing containing primary phosphates lowered the pH(H_2O) of the soil to pH 4.4.

Otherwise similar preparations were made in which 1 % calcium carbonate was incorporated. Thus the experiment could be made side by side with contamination replicates at a higher soil pH of 7.3.

After each preparation the soil was transferred to a plastic bag, mixed again by manipulating the closed bag, and put aside for a while to complete the other preparations.

Plastic pots were used of 1.9 kg soil-holding capacity. Two replicate pots were filled per treatment, and the remaining 0.2 kg portions dried and stored for soil analysis.

Plant cultivation was initiated by transplanting young plantain plants to the pots. They were cropped after six weeks. Then the pots were planted with young clover plants which were grown for eight weeks before being harvested. The third crop was from young grass plants transplanted to the pots and harvested four weeks later.

Between the successive crops the treated soils were removed from the pots, sieved to remove the roots and stubbles, refertilized with the basal dressing of N, P, K and Mg for sustainment of the next crop, and transferred back to the pots. Losses of soil were minimized during these operations.

The soil moisture content was frequently restored during the preparations and growth periods.

The plant material from the two replicate pots were combined to form one sample per treatment for the determination of the yield of DM and the content of the metal applied.

For plant and soil analysis a 3-g sample was transferred to a 100-ml volumetric flask, and 25 ml of nitric acid (7 mol/litre) were added. The mixture was left overnight, then gently boiled on a thermostated hot plate during four hours, made up to 100 ml with water, and filtered. In a 100-ml beaker, 50 ml of the filtrate was placed, and the liquid expelled on the hot plate. The residue was treated twice with 2 ml nitric and 2 ml hydrochloric acid in succession, each time expelling the acid on the hot plate. The dried residue was dissolved in 10 ml hydrochloric acid (1 mol/litre). The solution was centrifuged to remove suspended material, and analysed for the metal by atomic absorption.

Results and discussion

Soil analysis

The results of soil analysis are shown in Table 1. The increments from the preceding treatments agreed within 10 % with those calculated from the added levels.

The measured soil test levels were used as the independent variable for the interpretation of plant uptake and toxicity of the metals.

In the unlimed soil hydrolysis of the added metal salt lowered the pH. The effect was greatest with chromic sulphate, and reversed with the alkaline lead acetate.

In the limed soil the acidifying effect of the metal salts and the natural acidity of the soil were completely neutralized.

Acute toxicity of the metals in the soil

In the Zn, Ni and Cu series one or a few of the highest levels in the unlimed soil were destructive to the roots and caused rapid wilting and death of the plants. With Cd and Pb these levels of acute toxicity were not attained .

The transition from survival to death was abrupt and the number of survivors fixed from the beginning. A rough measure of the threshold level for root destruc-

Table 1. Results of soil analysis in mg metal/kg dry soils. Added levels were: 0, 1, 2, 4, 8, 16, 32 or 64 times 9.6 mg Zn, 4.3 mg Ni, 0.83 mg Cd, 15 mg Pb, 9.3 mg Cu or 15 mg Cr per kg dried soil.
Top: the unlimed acid soil of pH 4.4. Bottom: the limed soil of pH 7.3. The column at the right gives the soil pH measured at the highest level.

	Metal content (mg/kg dry soil)								pH
	0 ×	1 ×	2 ×	4 ×	8 ×	16 ×	32 ×	64 ×	
Unlimed soil									
Zn	9.5	18	27	48	79	155	312	608	4.2
Ni	2.1	5.7	9.8	24	36	62	140	279	4.3
Cd	0.2	0.9	1.8	3.2	6.7	13	28	53	4.4
Pb	13	31	46	86	160	289	570	980	4.7
Cu	3.8	12	20	37	61	128	238	531	3.9
Cr	5.3	21	38	81	150	285	542	952	3.7
Limed soil									
Zn	13.7	18	29	48	99	154	302	674	7.1
Ni	3.1	6.2	10	24	35	70	124	288	7.4
Cd	0.4	0.8	1.7	3.7	7.7	14	25	45	7.4
Pb	11.4	27	43	101	152	285	539	1062	7.2
Cu	4.0	12	19	29	88	140	314	514	7.3
Cr	6.0	22	40	92	151	257	618	845	7.3

tion is given by the concentration in the soil at the endpoint of the concentration curves for the surviving plants which will be shown in the next section.

The concentration curves

The results of plant analysis were recorded in the form of concentration curves relating the concentration of the metal in the shoot to the measured soil test levels of Table 1. For the wide ranges involved use was made of equal logarithmic scales.

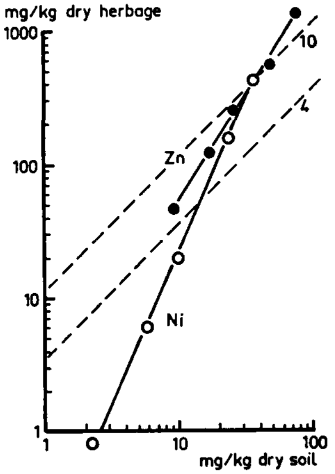


Fig. 1. Zinc and nickel in plantain and in the acid soil.

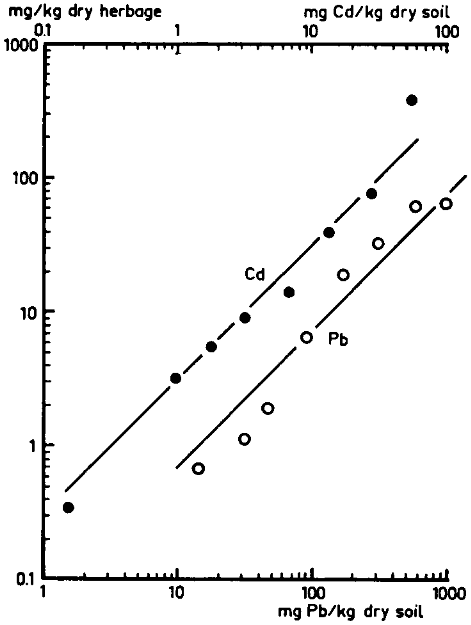


Fig. 2. Cadmium and lead in plantain and in the acid soil.

First, the curves obtained from the unlimed series were classified for shape and grouped as shown in Figs 1-3 for plantain as the experimental plant.

Straight lines were obtained for Zn and Ni which made angles with the soil concentration axis greater than 45 °. In the adopted type of graph this indicated greater relative increments in the plants than in the soil; the plant:soil concentration ratio increased with increasing levels of Zn or Ni in the soil. Taken at half the maximum soil concentration for survival (with this maximum considered as the soil concentration at the end-point of the curves) the ratios were around 10 for Zn, and 4 for Ni. These metals, known as being readily translocated to the shoot, displayed a disproportionally greater rise in shoot concentration as their level in the soil was raised.

For Cd and Pb (Fig. 2) straight lines were drawn through the points which were intentionally adjusted to make angles of 45 ° with the axes. The lines are situated at constant plant:soil concentration ratios of 3 for Cd and 0.1 for Pb. The results were assumed to agree with equal relative increments in plant and soil at a constant ratio as was also observed by Miles & Parker (1979) for Cd.

With Cu and Cr the curves were more complex with a flat portion in the lower range, and a break-through to the plant tops with the further increase in the soil test level (Fig. 3), of the type reported earlier by Cary et al. (1977) for Cr. At half the maximum soil concentration for survival the plant:soil concentration ratios were 0.2 for Cu and 0.05 for Cr. For Cu this was a minimum ratio at the point where the dashed line for 0.2 was tangential to the curve. For Cr the ratio 0.05 recurred at a lower level as a second intersect of the dashed line with the curve,

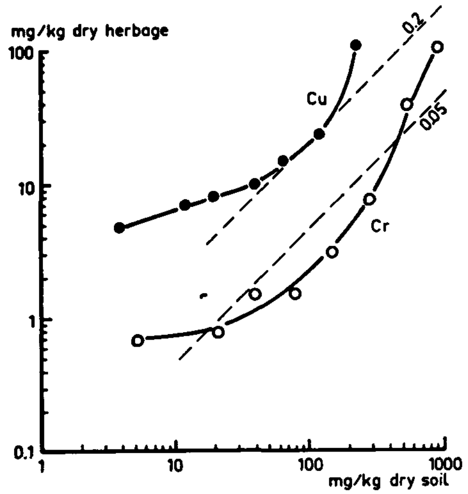


Fig. 3. Copper and chromium in plantain and in the acid soil.

with still lower ratios where the experimental curve runs below the 0.05 ratio and the first rise in soil concentration did not lead to an increase in the amount of the metal translocated to the shoot.

The curves obtained with clover and grass are not shown here because they were very similar in shape to those obtained with plantain.

With Cd, Pb and Cr all the plants survived so that the curves had eight records.

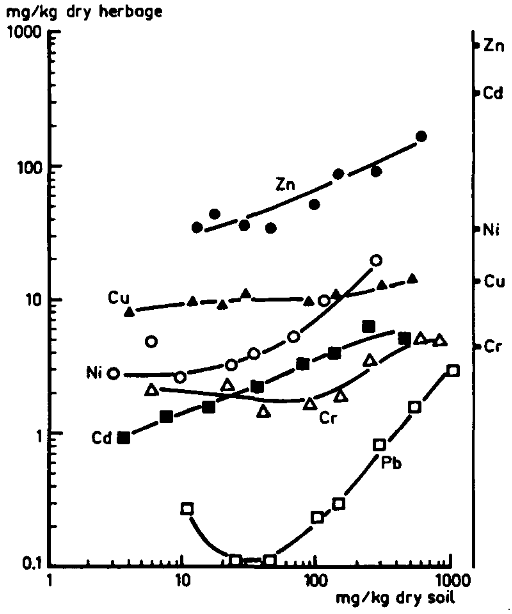


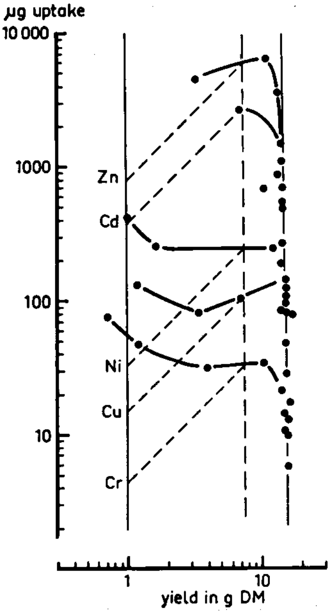
Fig. 4. Zinc, nickel, cadmium, lead, copper and chromium in plantain and in the limed soil of pH 7.3. Symbols at the right denote the inhibitive concentrations in the shoots as derived from the other series with no lime.

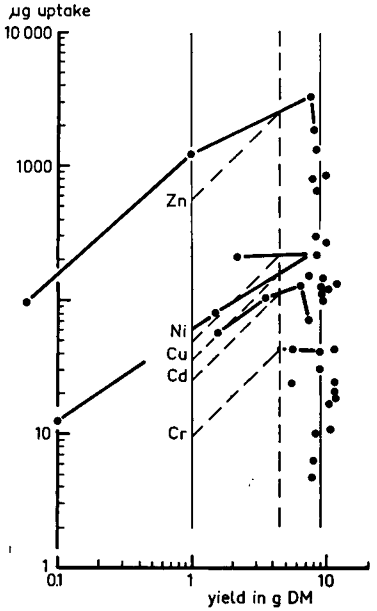
In the other metal series one or a few of the highest soil test levels were lethal with a correspondingly smaller number of records.

The order of decreasing plant:soil concentration ratios derived from the concentration curves (Zn, Ni, Cd, Cu, Pb, Cr) was close to that of decreasing translocation from the roots to the tops and removal from the soil by cropping, as gathered from the literature in the introductory section.

Plant and soil test levels in the series amended with calcium carbonate (soil pH 7.3) are shown in Fig. 4 for plantain. Complete survival produced eight records for all the metals. Liming flattened the curves and the plant:soil concentration ratios attained were of the order of hundredfold lower for Zn, Ni, Cd and Pb, and threefold lower for the more acidic Cu and Cr cations than they were on the acid soil. This and maintained survival of the plants at the highest soil test levels indicated poor accessibility of the metals to the root surface for uptake or injury when the soil was limed.

The symbols at the right indicate the herbage concentrations for 50 % repression of the growth as derived from the unlimed series (see below). They were attained on the limed soil only with Cu and Cr, and approached with Ni. These were the only series in which the plants were smaller at the high-metal side of the range. Once absorbed, these metals were as toxic to the growth as they were on the acid soil.





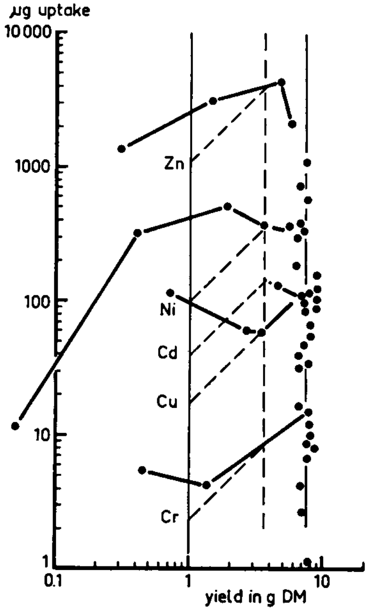


Fig. 5 (left). Uptake-yield curves for plantain on the acid soil.
Fig. 6 (middle). Uptake-yield curves for clover on the acid soil.
Fig. 7 (right). Uptake-yield curves for grass on the acid soil. In Figs 5-7 the solid vertical lines indicate the average maximum yield and the 1 g DM yield level, the dashed vertical lines half the maximum yield.

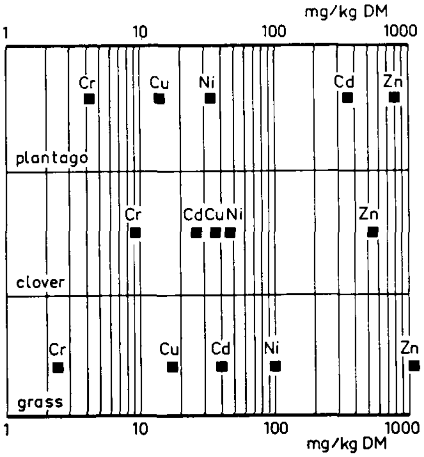


Fig. 8. The inhibitive shoot concentrations for 50 % yield depression in plantain, clover and grass.

Shoot concentrations for inhibition of the growth

The first response was a reduction in yield. Often the survivors remained healthy in appearance with no other symptoms than smaller plants.

In the toxic range between maximum growth and the point of mere survival the yield dropped progressively, presumably because the retardation of growth commenced earlier the higher was the initial rate of uptake.

Critical uptakes were assessed at the point at which the final yield had dropped to half the maximum produced at the lower unharmful metal levels in the soil. The uptakes were calculated from the yields and metal concentrations in the DM, and plotted against the yield of DM for construction of the uptake-yield curves of Figs 5-7.

As the uptakes increased with rising levels in the soil, the yield remained unchanged until the uptake had increased beyond a limit for maximum growth. From then the uptake ceased to increase, and the yield fell off with the further increase in soil concentration, thus concentrating the absorbed metal to still higher tissue concentrations until no survivors remained.

Thus each graph acquired the form of a comb with a vertical back of points clustering around the average maximum yield, and successive teeth extending towards the lower yields, each referring to one of the metals of which more was absorbed than were compatible with maximum growth.

The inhibitive tissue concentrations were estimated by marking the curves at 50 % of the average maximum yield, drawing a straight descending line through the mark and parallel to the diagonal between the axes towards the lower yield levels, and reading the uptake at its intercept with the ordinate erected at the yield level of 1 g DM. This was the uptake in µg metal/g DM, equivalent to the more conventional concentration unit of mg/kg DM, at the point at which the growth had been influenced to such an extent that the final yield had dropped to half the maximum produced at the nontoxic levels.

Suitable data for Pb were missing. At the highest level of 70 mg Pb/kg DM the yield was still at the maximum.

The approximations and interpolations underlying the translation of Figs 5-7 into inhibitive shoot concentrations were accepted in view of the large differences between the metals. The inhibitive tissue concentrations thus obtained have been assembled for the three plant species in Fig. 8.

The results show that the plants were most sensitive to Cr in the tops which was inhibitive at shoot concentrations below 10 mg Cr/kg DM, and least sensitive to Zn which became inhibitive at 600-1000 mg Zn/kg DM.

Tolerance to shoot Cr was least in grass and greatest in clover. More Ni in the shoot was tolerated by grass than by plantain, more Cu by clover than by the other two species. Plantain was particularly tolerant to Cd in the shoot which was confirmed by repeated plant analysis.

The inhibitive levels of Cd, Cu and Ni were intermediary with, in clover, small differences between these metals in inhibitive tissue concentrations.

Differential tolerance of plant species in the acid soil

Toxicity developed mainly on the acid soil of the unlimed series. The initial pH was 4.4, but pH decreased further with rising added levels of the metal salts (Table 1) which may have enhanced their uptake and toxicity to an unknown extent.

At 50 % yield reduction as the reference point for injury the soil pH was never below 4.2 except for Cr of which the larger dose decreased pH to 3.8 by stronger hydrolysis of the added chromic sulphate.

The large effect of liming the soil to pH 7.3 illustrated the importance of soil pH, the measurable effect of hydrolysis of the added types of metal salts and of the form in which the metals are added and affect soil pH (Dijkshoorn & Lampe, 1975).

Thus the tolerance rating which will be given below to the plant species and

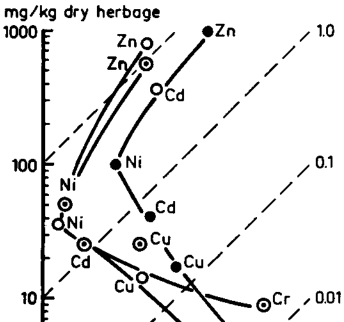


Fig. 9. Relation between the inhibitive shoot concentrations (50 % yield depression) and the concentration in the acid soil at which they were attained. Open dots: plantain. Encircled dots: clover. Solid dots: grass. The dashed lines indicate constant shoot: soil concentration ratios of whole powers of ten. The solid curves have been drawn to assist in visual appraisal of the change in inhibitive concentrations following substitution of the metals within each plant species.

metals with respect to the soil test levels applies only to the unlimed acid soil used and to the form in which the metals were added.

The tolerance rating for toxic plant levels was in our feeling independent of the experimental conditions. They agree with the toxic tissue test levels currently reported.

In Fig. 9 the inhibitive shoot concentrations of Fig. 8 have been plotted against the metal concentration in the soil at which they were attained. The latter values were obtained by marking the concentration curves of the type of Figs 1-3 at these plant concentrations, and reading off the corresponding soil concentrations. Data for Pb were missing because yield depression was not attained.

Between the equal logarithmic scales applied in Fig. 9 lines for constant plant: soil concentration ratios should run parallel to the diagonal between the axes. For orientation the four dashed lines have beeen drawn to indicate ratios of 10, 1, 0.1 and 0.01.

The plant species have been marked by joining the readings for the various metals. Hook-shaped curves resulted with the apex at low soil concentrations of Ni and Cd with shoot concentrations of the order of 50 mg/kg DM, and plant:soil concentration ratios of around 5.

For the restricted number of substitutions the curves could be applied as commensurable course lines for the effect of substitution of one test element for another on the inhibitive concentrations in the shoots and in the soil within each plant species. Only the point for Cd in plantain was situated far off from the curve for plantain which was due to the particularly high tolerance of this species to Cd in the tops.

This type of graph displays the essential effects of substitution of metals and plant species. The ordinates and abscissae indicate the inhibitive metal concentrations in the tissue and in the soil, the position of the points the plant:soil concentration ratios at the level for inhibition as a measure of the relative uptake at the inhibitive levels. Substitution without change in the inhibitive tissue concentraton but reducing the relative uptake will move the point horizontally to the right: the substituted element is equally toxic to the tissue but less harmful in the soil because more of it is needed in the soil for the uptake of the toxic quantity by the plant. Substitution with an increase in the tissue concentration for toxicity but without change in the relative uptake will move the point parallel to the diagonal between the axes towards higher concentrations: tolerance to the metal in the soil increases in direct proportion with the increased tolerance to the metal in the tissue. When the substitution effects proportional increases in relative uptake and in the tissue concentration for toxicity the point will move upwards in a vertical direction: the plant remains equally tolerant to the metal in the soil because its tolerance to the tissue metal is raised in proportion with the increase in uptake. All other shifts are the resultant of changes in relative uptake and tissue concentration for toxicity.

The upper branch of the curves is directed towards Zn with a twofold greater relative uptake than Ni, but tolerated at a twentyfold higher concentration in the shoot. Hence the permissible soil concentration of Zn was some ten times higher than that of Ni.

The downward trending lower branch is directed to Cr which bcame toxic at a ten times lower concentration in the shoot than Ni, but had a hundredfold lower relative uptake. Hence the permissible soil concentration of Cr was about ten times higher than that of Ni.

Differential tolerance of the plant species can be elucidated by the shift of the point for one metal with substitution of one species for the other.

For instance, clover-for-plantain involved no change in the relative uptake by the shoot of Cr: the point moved parallel to the diagonal. But twice as much Cr in the shoot was tolerated by clover so that the tolerance to soil Cr was two times greater in clover than in plantain.

Cr was more toxic in the shoots of grass than in clover, but less readily absorbed by grass so that clover and grass were about equally sensitive to Cr in the soil.

Grass-for-plantain moved the point for Ni parallel to the diagonal towards higher concentrations: the relative uptake remained unchanged, but the tissue concentration for inhibition was three times higher in grass. Hence, three times more Ni in the soil was tolerated by grass.

Plantain tolerated ten times more Cd in the tissue than grass. Plantain-for-grass raised the relative uptake of Cd by tenfold. Hence both species were about equally tolerant to Cd in the soil.

Grass was a little more tolerant to tissue Zn than were plantain and clover, but distinctly more tolerant to soil Zn because relatively less of the soil Zn was taken up by the shoots of grass.

Cu was tolerated by clover at a higher tissue concentration than by grass, but grass tolerated a higher soil concentration of Cu because disproportionally less of the soil Cu was taken up by the grass.

Tissue Cu was about equally toxic to grass and plantain, but soil Cu was less toxic to grass as a result of the smaller relative uptake by grass.

Greater tolerance of grass to most of the metals in the soil was expressed by the position of the curve for grass at soil test levels higher than the range occupied by the curves for plantain and clover. This was partly due to higher tissue concentrations for toxicity, for the other part to smaller relative uptakes.

At this point it should be recalled that the metals were added for once to the soil. Prior to grass, the preceding crops had removed some small part of the supply. Integrated for the first two crops the uptakes by the shoots were of the order of 4, 1, 0.5, 0.2 and 0.05 % of the supply inhibitive to the growth for respectively Zn, Ni, Cd, Cu and Cr. Since the whole plants were removed from the soil, the actual degree of exhaustion could not be estimated. For Zn which distributes readily over the various plant parts, it might have been of the order of 10 % at the time the grass was planted, for the other metals probably less.

It seems unlikely that this small depletion, which would remain within the error of soil analysis, could have been responsible for the threefold greater tolerance of the grass to the soil test levels, unless plant availability concerns a small fraction of the added metal which is not quickly restored after its removal by plant uptake.

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